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A COMPARATIVE STUDY OF PRECIPITATION TIME AND TEMPERATURE FACTORS AFFECTING BLOOD AND URINE CALCIUMS‡

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Many variations of time and temperature have been described for the precipitation of calcium oxalate in the determination of blood and urine calciums. Kramer and Tisdall's[1] original article precipitated for 30 minutes or overnight at room temperature and other authors have suggested four hours and 16 hours[2] at room temperature and overnight in the refrigerator. More recently Ribeiro de Souza[14] suggested three minutes at 63° C. and Rappaport[15] precipitated five minutes at 56° C. A need is realized for a comparison of accuracy and rapidity on these suggested periods and temperatures.

By means of the flame photometer, a rapid estimation of serum Na and K is possible, whereas the instrumentation is not adequate at the present time to permit its use for calcium. In our laboratory a Beckman DU spectro-flame photometer is in use daily, but the blood and urine calciums remain a titrimetric procedure to afford experience to our students.[1] The expense of a flame photometer and lack of experienced personnel may long limit small laboratories to the use of these titration methods. The available precipitation methods for serum calciums require at least four hours or usually overnight. This is too long when a doctor must decide whether a low calcium is a factor in the cause of convulsions. Since this problem is often encountered in newborns and prematures, management of such patients begins without benefit of calcium levels. It is the purpose of this article to compare the overnight calcium precipitation period to short incubation precipitation periods for both blood and urine calciums.

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‡ 2nd Award ASMT Convention, 1954, Miami Beach, Florida.

Serum Calcium Studies

The serum calciums were precipitated as calcium oxalate, centrifuged and the excess oxalate washed out with 2% ammonium hydroxide. The washed calcium oxalate was dissolved in sulfuric acid and titrated at 70° C. with .01 N potassium permanganate.[1, 2]

Comparative analysis of the same serum samples in duplicate were carried out with varying precipitation periods and temperature, but the methods of washing and measuring the oxalate were the same throughout. Control analyses of standard calcium carbonate (Iceland spar) solutions paralleled the serum analyses at every step to insure accuracy. Blank analyses were also run to correct for any calcium in the reagents which was always a titratable amount (around .04 to .06 ml. of .01 N $KMNO_4$). Acid washed[2] glassware and double distilled water were used. Human serum was obtained from clotted blood, and samples of 2 ml. were analyzed in duplicate according to the techniques noted.

Comparative results are presented in Table I. It is seen that excellent agreement was obtained between the overnight precipitation and the 15 minute precipitation at 56° C. The average deviation was ±.039. Büll[6] found that calcium could be completely precipitated in some sera in ½ hour, and other sera required 16 hours depending on viscosity and protein content of

TABLE I

Comparison of Serum Calcium Values (mgs/100 ml.) with Varying Precipitation Conditions and Proteins Observed.

Precipitated Overnight in Refrigerator a.	Precipitated 15 Minutes at 56° C. b.	Difference b-a	PROTEIN GRAMS/100 ML.		
			Total	Alb	Glob
9.44	9.40	—.04	3.2	.5	2.7
9.50	9.50		6.1	3.0	3.1
9.50	9.50		6.0	3.2	2.8
9.50	9.50		5.6	3.1	2.5
9.56	9.60	+.04	5.6	2.0	3.6
9.80	9.85	+.05	6.4	3.4	3.0
9.90	10.0	+.10	6.2	3.3	2.9
10.0	10.0		6.3	2.6	3.7
10.02	10.0	—.02	7.3	4.1	3.2
10.25	10.3	+.05	7.2	3.6	3.6
10.50	10.35	—.15	7.2	3.6	3.6
10.45	10.50	+.05	7.0	2.9	4.1
10.50	10.55	+.05	6.8	3.4	3.4
10.60	10.50	—.10	6.4	4.0	2.4
10.92	10.90	—.02	7.1	4.5	2.6
11.06	10.94	—.12	7.3	4.0	3.3
11.10	11.10		8.3	4.4	3.9
11.40	11.30	—.10	10.0	2.2	7.8*
11.60	11.60		7.7	5.1	2.6
11.75	11.80	+.05	11.8	1.6	10.2*
11.90	11.80	—.10	7.5	4.4	3.1
12.10	12.10		.....	.....	.....
12.40	12.30	—.10	.....	.....	.....
Mean [3] 10.54	10.55	Avg. ±.039	.....	.....	.....

[3] 47 cases were studied but due to lack of space, only 23 are presented. The mean and average difference were based on all 47 cases.

* Multiple Myeloma.

the sample. The data collected, including two cases of multiple myeloma, revealed no such trend even on proteins varying from 3.2 gms% to 11.8 gms%.

Other incubation periods were tested with representative data shown on Table II. The average values encountered in the 56° C. periods were 2 to 4% lower than the 15 minute or overnight precipitation periods. The 30 minute precipitations at room temperature[1, 13] gave values of 3 to 5% lower. Some authors advocate the direct precipitation of calcium with EDTA, but no comparisons were made since this procedure is still in the developmental stage and has not yet been proven.[5, 7, 9, 12]

TABLE II

Comparison of Serum Calciums (mgs/100 ml.) in Varying Precipitation Periods

Precipitated: Overnight in Refrigerator	15 Min. at 56° C	2 Min. 56° C.	3 Min. 56° C.	5 Min. 56° C.	10 Min. 56° C.	13 Min. 56° C.	17 Min. 56° C.	25 Min. 56° C.	30 Min. Room Temperature
9.60	9.70				9.30				
9.80	9.85								9.60
9.90	10.00	9.60	9.60	9.60					
10.10	10.10					9.90			
10.25	10.30	10.00	10.00	10.11					
10.45	10.50					10.20	10.30		
10.45	10.50					10.20	10.30	10.20	
10.50	10.42			10.05				10.05	10.0
10.70	10.70			10.35				10.45	10.35
10.92	10.90	10.60							
11.40*	11.30	11.0							
11.75*	11.80	11.3							11.25

* Multiple Myeloma.

Urine Calcium Studies

The estimation of urine calcium is more difficult than the estimation of serum calcium. The tendency of urates and other organic materials to precipitate along with calcium oxalate interferes in the subsequent titration. The precipitation of calcium oxalate was carried out at pH 3.0-3.3 to prevent coprecipitation of magnesium, phosphate, and other materials. A comparison is made between the direct acidimetric titration,[8] and the modified oxidimetric titration[1, 2]; and between the 3 hour precipitation at room temperature[4] and 15 minute precipitation at 56° C. of calcium oxalate.

Procedure

Fifty ml. of 24 hour urine collections were boiled with concentrated nitric acid until clear. When cool these were diluted to 100 ml. with distilled water and 2 ml. aliquots were pipetted into eight 15 ml. pyrex centrifuge tubes. Four blank reagent tubes each containing 4 cc. of distilled water and eight control

[2] Cleaning solution, 100 grams sodium dichromate dissolved in water to 9 lb. technical grade concentrated sulfuric acid.

tubes of standard calcium carbonate solutions (1 cc = .2 mg Ca) were prepared and paralleled the urine analyses from this point on. All the tubes were then divided into four groups.

Group A—This group consisting of one blank tube, two standard tubes and two urine aliquots received one ml. of saturated ammonium oxalate. A drop of .04% brom-cresol-green was used to adjust all tubes to pH 3.0-3.3 by adding 50% ammonium hydroxide to faint blue green and 5% HCl to definite light yellow. The tubes were allowed to precipitate at room temperature for 3 hours and were then centrifuged and decanted. The precipitate was washed with 2% ammonium hydroxide to eliminate the excess oxalate. The washed calcium oxalate was dissolved in 1 N sulfuric acid and titrated at 70° C. with .01 N potassium permanganate.

Group B—One ml. of saturated ammonium oxalate was added to the blank tube, two standards, and two urine aliquots composing this group. After adjusting the pH to 3.0-3.3 as above, the tubes were allowed to precipitate 3 hours at room temperature. At the end of this time, the tubes were centrifuged, decanted, and washed with 0.5% ammonium oxalate. After drying, they were heated to 550° C. in a muffle furnace for one hour to convert the calcium oxalate to carbonate. The carbonate was dissolved in hot boric acid and after adding 2 drops of .04% brom phenol blue, all tubes were titrated with .01N HCl.

Group C—One blank, two standards and two urine aliquots received 1 ml. of saturated ammonium oxalate and after adjusting the pH to 3.0-3.3, were incubated 15 minutes at 56° C. At the end of this time they were centrifuged and decanted. The precipitate was washed with 2% ammonium hydroxide, centrifuged and then dissolved in 1 N sulfuric acid preparatory to titrating with .01 N potassium permanganate.

Group D—The last five tubes (one blank, 2 standards, and two urine aliquots) received one ml. of saturated ammonium oxalate. After being brought to a pH 3.0-3.3, they were precipitated at 56° C. for 15 minutes. The precipitate was then washed with .5% ammonium oxalate, dried and heated in a muffle furnace for 1 hour at 550° C. The carbonate was dissolved in hot boric acid and titrated with .01 N HCl.

Table III shows a comparison of nine urine calciums each treated in the four different ways. It is shown that excellent agreement was obtained between the two precipitation periods and the two titration methods both in the urine aliquot titrations in Table III and in the precision of standards presented in Table IV. The advantage of this 15 minute precipitation at 56° C. and the permanganate titration (method used on Group C) is its

[4] Previous work comparing 3 hours precipitation at room temperature and overnight precipitation in the refrigerator gave excellent agreement.

TABLE III

Determination of Calcium in the Urine
The values are expressed in mg. of Ca per urine aliquot

Group A	Group B	Group C	Group D	Albumin Present
.41	.40	.42	.42	none
.38	.36	.39	.36	none
1.11	1.09	1.00	1.11	1.0 gm/l
.24	.21	.23	.23	none
.65	.65	.68	.67	none
.50	.53	.52	.52	none
.47	.48	.48	.50	.2 gm/l
.79	.80	.80	.79	none
1.01	1.01	.99	1.01	none

rapidity without sacrifice of accuracy. In addition permanganate titration obviated the necessity of a muffle furnace and for all these reasons, the procedure used on Group C is the method of choice.

Results of titration values on standard tubes paralleling urine aliquots are shown in Table IV. Theoretical recovery of standard was 1.0 cc. of .01 N HCl or 0.1 N $KMNO_4$.

TABLE IV

Comparison of Standard Values Obtained on Four Techniques

	Group A	Group B	Group C	Group D
Mean	1.04	1.02	1.008	1.009
Standard Deviation	± .011	± .033	± .028	± .034

Summary

1. Data was presented on the comparison of 15 minute precipitation at 56° C. versus overnight precipitation in the refrigerator of calcium oxalate in serum calciums with the conclusion that excellent agreement is attained and the rapidity of the 15 minute precipitation makes it the method of choice.
2. The shorter and longer precipitation periods at 56° C. gave results 2-5% lower than the 15 minute period.
3. For urine calciums, comparison is made between the 15 minute precipitation period at 56° C. and 3 hour room temperature precipitation. Comparison is also made between acidimetric and oxidimetric titrations again with excellent agreement.
4. For rapidity and accuracy, the method of choice for urine calciums is the 15 minute incubation at 56° C. and the oxidimetric titration which eliminates the necessity of a muffle furnace.

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WHAT IS RESEARCH?

"Research" is a high-hat word that scares a lot of people. It needn't. It is rather simple. Essentially, it is nothing but a state of mind—a friendly, welcoming attitude toward change. Going out to look for a change instead of waiting for it to come. Research, for practical men, is an effort to do things better and not to be caught asleep at the switch. The research state of mind can apply to anything: personal affairs or any kind of business, big or little. It is the problem solving mind as contrasted with the let-well-enough-alone mind. It is the composer mind instead of the fiddler mind. It is the "tomorrow" mind instead of the "yesterday" mind.

—Charles Kettering

DEVELOPMENTS AND TRENDS IN THE SERODIAGNOSIS OF SYPHILIS[1]

GENEVIEVE W. STOUT, M.A.,[2] AD HARRIS[3] and SIDNEY OLANSKY, M.D.[4]

All laboratory diagnostic procedures undergo frequent changes as the result of refinements of existing technics and the development of new methods. The serodiagnosis of syphilis is no exception. During the past fifteen years, we have seen the lipoidal antigens used in well established tests for syphilis replaced with cardiolipin antigens, tube tests are being supplanted by slide tests, qualitative tests on reactive sera are being supplemented by quantitative reports, and changes in terminology have been advocated for reporting test results. Efforts directed toward the improvement and standardization of test performance have been greatly increased and are showing encouraging results.

Developments in the serology of syphilis have been continuous since the first tests were performed by Wassermann and his associates in 1906.[1] Beginning with the publication of the Kahn test[2] in 1922, numerous serologic tests were developed in the United States. International and national evaluation studies were carried out to determine the efficiency of various testing methods. The Washington Serology Conference was held in Washington, D. C., in 1941, under the auspices of the United States Public Health Service for the purpose of evaluating original methods. The results[3] substantiated the reliability of the more generally used American methods; namely, the Eagle, Hinton, Kahn, Kline, and Mazzini flocculation tests, and the Eagle and Kolmer complement fixation tests. Lipoidal antigens (crude extracts of beef heart) are used in all these procedures.

In 1941, another phase in the development of syphilis serology began with the isolation by Pangborn[4,5] of the substances responsible for the reactivity of alcoholic extracts of beef heart in tests for syphilis. Pangborn isolated and purified a nonnitrogeneous phospholipid from beef heart which she called cardiolipin. By combining cardiolipin with suitable proportions of purified lecithin, antigens were compounded which could be used in both complement fixation and flocculation tests. These antigens will be referred to as cardiolipin antigens.

Some of the significant reports regarding the utilization of cardiolipin antigens in existing test procedures and in new tests are listed below in chronological order:

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[1] From the Venereal Disease Research Laboratory, Division of Venereal Disease, United States Public Health Service, P. O. Box 185, Chamblee, Georgia.
[2] Bacteriologist, USPHS, Venereal Disease Research Laboratory, Chamblee, Georgia.
[3] Associate Director, Venereal Disease Research Laboratory, Chamblee, Georgia.
[4] Director, Venereal Disease Research Laboratory, Chamblee, Georgia.

CARDIOLIPIN ANTIGENS IN TESTS FOR SYPHILIS

Year	Authors	Test	Report on Use of Cardiolipin	Recommended Method	Test
1944	Harris, Portnoy [6]	Kolmer C.F.*	X		
	Maltaner,				
	Maltaner [7]	New York C.F.*		X	
1946	Rein, Bossak [8]	Rein-Bossak			X
	Kline [9]	Kline		X	
	Harris, et al. [10]	VDRL Slide			X
	Kahn, et al. [11]	Kahn	X		
1948	Stuart, et al. [12]	Hinton	X		
	Kahn, et al. [13]	Kahn	X		
	Kolmer, Lynch [14]	Kolmer	X		
	Harris, et al. [15]	VDRL Tube			X
1951	Mazzini [16]	Mazzini		X	
	Ipsen [17]	Hinton		X	
1953	Kolmer [18]	Kolmer		X	

* Complement fixation.

The change in the United States from the use of serodiagnostic tests for syphilis employing lipoidal antigens to the use of tests with cardiolipin antigens has taken place in the past ten years. In 1946, Harris and Mahoney,[19] from the Venereal Disease Research Laboratory, United States Public Health Service, Staten Island, New York, reported at the American Public Health Association that "cardiolipin as an antigenic substance in tests for syphilis has been employed only in a few laboratories and for a relatively short period." In 1949, Arnold and Mahoney[20] reported from the same laboratory that "cardiolipin as an antigenic component has now assumed a major role in the serology of syphilis." In 1954, with Hinton, Kline, Kolmer and Mazzini recommending the use of cardiolipin antigens in these procedures, the only commonly used test in the United States in which a lipoidal antigen is still favored is the Kahn test.

With the report of Nelson and Mayer[21] in 1949, on the Treponema Pallidum Immobilization test, interest was revived in serodiagnostic tests using Treponema pallidum as antigen. Specific agglutination of Treponema pallidum as described by Cain,[22] Hardy and Hollander,[23] and McLeod and Magnuson.[24] Nelson has more recently[25, 26] reported on an Adhesion-Disappearance Phenomena which may have practical application. D'Alessandro and Dardani[27] have reported in the American literature on the isolation and purification of a protein antigen from the Reiter treponema. Numerous investigative studies are in progress and the results should be extremely interesting.

There are therefore three types of tests for syphilis to be considered, using lipoidal, cardiolipin and treponemal antigens. Lipoidal and cardiolipin antigens are ordinarily used in the tests for syphilis as conducted in public health and clinical laboratories. Tests with treponemal antigens are, at the present time, used primarily in research laboratories as experimental proce-

dures in controlled studies. The Treponemal Pallidum Immobilization test, however, is being used in a very limited number of laboratories as a diagnostic aid in selected study cases.

Lipoidal antigens are still used in tests for syphilis in many laboratories. The Kahn test employing a lipoidal antigen is quite generally used in the United States and throughout the world. When properly performed and controlled, it has, and will continue to be a reliable test procedure. The Eagle Flocculation and complement fixation tests use lipoidal antigens, but since Eagle is no longer working in the field of syphilis serology, there is no control for the antigens used in these tests, and relatively few laboratories continue to employ them. The use of lipoidal antigens in the Hinton, Kline, Kolmer, and Mazzini tests is currently practiced in some laboratories, but as experience is gained in the use of cardiolipin antigens in these procedures, this will no doubt decline. Many comparative studies have been made, and should be continued before changes are adopted.

There is a very definite trend toward the adoption of tests with cardiolipin antigens. The main advantages lie in the reproducibility of the antigens and the fact that tests using these antigens are more specific than those using lipoidal antigens. Antigens composed of cardiolipin and purified lecithin can be assembled using known amounts of these components and standardized both chemically and serologically. Cardiolipin and cholesterol can be used in exact amounts, but since different lots of lecithin are not absolutely reproducible, variations within narrow limits are necessary for serological standardization.

The increased specificity of tests using cardiolipin antigens is probably due to the fact that many of the antigenic reactive impurities present in lipoidal antigens are not present in cardiolipin antigens. The nonspecific factor found in some Kolmer lipoidal antigens is absent in the cardiolipin antigen used in the Kolmer test.[6] It should be emphasized, however, that while tests with cardiolipin antigens have been shown to give fewer false positive or nonspecific reactions, these will not be entirely eliminated.

The VDRL Slide test[10] is one of the new methods which was developed to make use of cardiolipin and lecithin as antigen components. The test technic is simple, using only two reagents, and the antigen emulsion can be used immediately after it is prepared and throughout the day. Tests are rapidly performed with multiple testing on glass slides, a short rotation period, and microscopic reading. Many comparative studies have shown the test results to be reliable, both in the United States and other parts of the world. In central America, the use of the VDRL Slide test has proved to be a very practical procedure[28] with a population group which has a high false positive potential. At present, the VDRL Slide test is the most widely used of the

various tests using cardiolipin antigens in examining blood specimens for syphilis. The same basic antigen emulsion may be used in a tube test with serum,[15] and in a spinal fluid test.[29] The spinal fluid test is used extensively and has been shown to be comparable in sensitivity to the Kolmer complement fixation test,[30] and other tests.[31]

In 1948, a Subcommittee to Develop Reference Methods for Syphilis Serology, was appointed by the American Public Health Association. The function of the Committee as stated in their report[32] was to "deal with a confusing situation in the realm of syphilis where there existed a number of laboratory methods and a considerable lack of understanding on the part of practicing physicians about the limitations of the various tests in use, with a consequent need for standard or approved methods that could be used for reference." In 1951, this committee reported a technic for a "Standard Method for Performing a Microflocculation Test for Syphilis" which was published in the American Journal of Public Health.[32]

The reactivity level of the antigen to be used in this procedure has not been finally established. At the annual meeting of the American Public Health Association in 1953, the committee reported that a cooperative clinical study had been undertaken in cooperation with the World Health Organization which would provide necessary information for selecting an optimal level of sensitivity for this reference antigen. When the reference method is fully established, it is expected that it will be part of the Diagnostic Procedures and Reagents of the American Public Health Association and provide a standard method against which other procedures may be evaluated.

The test is very similar to the VDRL Slide test. The antigen emulsion is prepared in the same manner, and the test proper consists of a quantitative reaction performed with serum and antigen emulsions used in amounts which are eighty percent (80%) of those used in the VDRL Slide test. Test results are read as either "reactive" or "nonreactive," and "reactive" results are reported in terms of the highest dilution of serum which causes clumping of antigen particles.

Serologic tests for syphilis using treponemal antigens are not, at the present time, in a position to be used as routine testing procedures. Investigative studies with the Treponema Pallidum Immobilization (TPI) test of Nelson,[21] published in 1949, are being made in a number of laboratories in the United States and other countries. A bulletin[33] recently issued by the Bureau of Medicine and Surgery, United States Navy Department, lists twenty-eight (28) laboratories in twelve (12) different countries as now perfoming this test.

In the TPI test, the patient's serum is combined with a

suspension of virulent treponema pallidum and complement under specified conditions for a given period of time. At the end of this time, usually eighteen (18) hours, the percent of treponemes which were immobilized is determined as an index to the presence or absence of immobilizing antibody in the serum of the patient. This antibody is known to be present in serum of patients with syphilis and other treponemal infections, but is not ordinarily present in their absence. The immobilizing antibody has been shown to be distinct from the reagin,[21,34] determined by serologic tests using lipoidal and cardiolipin antigens. Numerous studies have contributed to the knowledge of the behavior of the TPI test in the different stages of syphilis,[25,34,35,36] both treated and untreated. The test is considered to be of value in differentiating false positive reactions in standard tests for syphilis from syphilitic reactions.[25,37,38,39] Known test limitations must be taken into account in evaluating test results. The use of the test is restricted by the fact that it is technically difficult, time consuming, and expensive to operate.

Other tests using treponemal antigens are even less clearly defined at this time. The Adhesion-Disappearance Phenomena described by Nelson,[25,26] specific agglutination of treponema with syphilitic serum reported by Cain,[22] Hardy and Hollander,[23] McLeod and Magnuson,[24] and the Italian studies of the isolation and purification of the protein antigen of the Reiter syndrome[27] are important.

There is a very noticeable trend toward the adoption of slide test procedures. Reliable results can be obtained with multiple tests being performed on a single slide. By substituting slide tests for tube tests, it is possible to examine large numbers of specimens in less time, with less glassware, and with fewer people.

The results of quantitative reactions in serologic tests for syphilis have become an important aid to the physician in the evaluation of treatment response in the modern therapy of syphilis. Consequently, the quantitation of all reactive sera is becoming a routine practice. The majority of State Public Health Laboratories in the United States[40] perform a quantitative test on all sera which are "positive" or "reactive" in qualitative tests. The proposed APHA Reference "Microflocculation Test for Syphilis"[32] is a slide quantitative method.

In reporting test results, the newer test procedures[8,10] and modifications of existing procedures[9,16] use the words "weakly positive" in place of "doubtful." At a meeting of the National Serology Advisory Council in 1953, the resolution was adopted that the terms "reactive," "weakly reactive," and "nonreactive" would be used in lieu of "positive," "doubtful," and "negative."

This practice has been adopted in several State Public Health Laboratories.

The reliability of the results of serodiagnostic tests for syphilis depends not only on the use of testing methods of proven worth but also on the proper performance of the tests. Test results are no better than test performance. This has been clearly demonstrated by the numerous evaluation studies in syphilis serology which afford a comparison of results obtained in participating laboratories with those of a control laboratory. There are International, National and State programs for the standardization and improvement of test performance. The programs all stress the same basic principles:

1. Strict adherence to the technic recommendations of the originator of the test.
2. The use of standardized reagents.
3. The use of adequate controls, including the use of control serums of known reactivity.
4. Reporting of results as specified for the procedure used.

In the United States the National Program of the United States Public Health Service has proved effective and the quality of test performance in State Public Health Laboratories is, in general, satisfactory. This program has included the training of personnel, improvement in the quality of test antigens and reagents, consultative services, technical reviews, and National Serology Evaluation Surveys.

State programs are, in operation, patterned after the National Program. Thirty-eight (38) States in the United States of America now have intrastate evaluations[40] of syphilis serology. In those States where it has been possible to supplement these studies with training, laboratory visitation, and consultative services, the improvement shown in test results has been very gratifying. Some States have made standardized reagents available, either free or at cost, and in at least one instance, selected lots of commercial antigens are certified as having "standard" reactivity. Field refresher courses and workshops have been held to demonstrate the correct performance of published test technic used in a particular area and to discuss the problems which arise in connection with syphilis serology. The cooperation of the State Societies of Medical Technology in promoting and attending these courses has been helpful. Despite the improvements noted, there are still many laboratories reporting unreliable test results, and efforts must be continued and increased if we are to have dependable syphilis serology in all laboratories.

Summary

The developments and major changes in the serodiagnosis of syphilis in the past fifteen (15) years has been reviewed; the

trend is toward the use of tests employing cardiolipin antigen. Rapidity of test performance and economy of operation has resulted in the wide use of slide tests. Quantitative serology is becoming a routine procedure. Programs designed for the improvement and standardization of syphilis serology are obtaining promising results.

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GENERAL REMARKS ABOUT THE DIAGNOSIS OF THE ZOONOSES*

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Habitation close to and occupational contact with animals exposes rural people to certain diseases that are primary in animals and secondary in man. The word "zoonosis," originally chosen by Virchow, is applied to diseases of animals that secondarily are contagious to man. Certain epizootic or enzootic infections of domesticated animals are particularly important, and equally so are the bacterial, viral and protozoan infections conveyed by contact or by vector in forest and field adjacent to human dwellings. At least 89 infections of domestic and wild vertebrates are a potential threat to man's health. The care, breeding, frequently the butchering or consumption of flesh from diseased or invisibly infected animals offer unlimited opportunities for transfer of the infective agents. The bacterial, fungous, protozoan, viral and helminthic infections may invade the human body by any of the several routes—through the mouth, the skin or mucous membranes and the respiratory tract. Direct contact with diseased animals or animal products or consumption of animal products contaminated during processing and delivery are the most common modes of transmission. The infective agent may also be transmitted through an intermediary vector. In this case man, usually in rural areas because of their proximity to wild animal reservoirs, acquires the disease without ever having had contact with the animal host. Reservoirs of infection in lower animals now dominate the problems of zoonoses in their relation to human welfare. Because the infective agents living in animals are quite ordinarily totally inconspicuous, the real problem of suppressing or eradicating zoonoses is much less a problem of wiping out overt disease than of discovering the true reservoir and frequently of dealing with ineradicable mammalian or bird reservoirs. A cursory glance at the available statistics on zoonoses has, until recently, failed, with few exceptions, to impress public health workers with the extent of the hazards confronting people leading a rural life. Inadequate reporting due to the lack of readily accessible diagnostic and medical facilities contributes greatly to the difficulties of knowing the exact magnitude of the problems throughout the world.

* Adaptation of an address, "Animal Diseases and Human Welfare," given at the Annual Convention, American Society of Medical Technologists, on June 24, 1952, at Portland, Oregon.

It will remain so until trained personnel—veterinarians and auxiliary workers—are assigned the task of taking accurate animal censuses and collecting complete data on animal diseases. Economically and as sources of infection to the country dweller, losses due to morbidity of domesticated animals may heavily outweigh the losses due to death (Meyer, 1942, 1953a; World Health Organization, 1951, Steele, 1953a and b; Kaplan, 1953).

In Table I the most important zoonoses are listed. It is not necessary here to detail specific aspects of epidemiology, mode of transmission and pathways of infection. They can be found in other recent publications (Hull, 1947; Meyer, 1948, 1953a; U. S. Public Health Service, 1953). Diagnostic procedures which, with increasing frequency, will engage medical technologists will be summarized. After a brief discussion of certain zoonoses, some of the steps required for diagnosis will be mentioned. The recently published World Health Organization monograph, "Advances in the Control of Zoonoses" (World Health Organization, 1953) and the excellent pamphlet, "Specimens for Laboratory Examination," issued by the Animal Research Station, Department of Agriculture, Wallaceville, New Zealand (1951) have been used freely as a basis for these remarks:

Bovine Tuberculosis. Epidemiologic studies in Great Britain and Denmark give reason for concern about the spread of the bovine tubercle bacillus to rural people. It is responsible not only for the extrapulmonary forms contracted through consumption of raw milk, but also for pulmonary tuberculosis in man. Destruction of tuberculous cattle and pasteurization of milk have reduced the incidence in areas where these measures have been taken, but this disease continues in others where infected cattle, contaminated barns and raw milk serve as vehicles for its spread.

Though the bovine tubercle bacillus is doubtlessly of less importance to the human population of the United States than of some other areas, it is important to know the steps required for the isolation, identification and typing of tubercle bacilli:

(1) Tubercle bacilli can be isolated on Löwenstein's or Besredka's medium (Jensen, 1953).

(2) Sputum, urine, stomach washing, spinal fluid, exudates and tissues, after proper treatment to destroy the contaminant bacteria, are seeded on the solid medium.

(3) Typing depends on the characteristics of the colonies. The growth of most human types of tubercle bacilli is greater in amount than that of the bovine type. For this reason the human type is called eugonic, the bovine dysgonic. Several deviations from the two well-defined types have been noted, and subtypes or atypical strains have been described. One must obviously guard against errors in identifying acid-fast saprophytes resem-

bling tubercle bacilli. It has been reported that when p-aminosalicylic acid is added to the medium true tubercle bacilli cannot multiply whereas saprophytes are not affected.

(4) Virulence is measured by inoculating test material into rabbits. On intravenous inoculation of 0.01 mg. of dysgonic tubercle bacilli the animals usually die of acute generalized tuberculosis within 30 days after the inoculation. The human type in this dose does not produce tuberculosis in the rabbit.

(5) Tubercle bacilli are sought in milk through microscopic examination of deposits obtained through centrifugation and with the biologic test on guinea pigs (Stuart, 1953).

Brucellosis. Inadequacies in reporting make it difficult to see the true picture of the illness, misery and economic loss caused by brucellosis. Economic losses attributable to brucellosis are reflected in lowered capacity for work and actual illness of people and in the abortions, infertility and decline of milk production among infected animals. Unfortunately these losses fall most heavily on economically underdeveloped countries of Latin America and the Mediterranean area. Through the energetic efforts of WHO and FAO, the work of livestock groups, the dairy industry, veterinarians, public health authorities and physicians has been coordinated and a new outlook—complete eradication of bovine brucellosis rather than adaptation to it—has been adopted. In carrying out these recently coordinated efforts it is frequently impossible to make diagnostic tests or they can be executed only with great difficulties, and this impedes elimination of infected animals. Evidence now available indicates that the seroagglutination test must be interpreted on a herd basis, not on an individual animal basis. Caprine and ovine brucellosis, of tremendous social importance in the rural life of a great many countries, would also lose ground through such a program. The environment of human habitations where infected goats have been housed for decades is thoroughly impregnated with the infective agent. If an eradication program of testing and slaughtering is followed, a primitive goat industry could meet brucellosis only by replacing their stock through importation. Such a measure is not compatible with the economy of the countries most heavily burdened by this disease. A suitable vaccine for use in sheep and goats has not been developed, but several laboratories have developed preparations that show promise of protective ability.

The laboratory procedures in the diagnosis of human brucellosis and their limitations have been amply described in many publications, and one merely recalls some highlights:

(1) Blood culture. An unequivocal diagnosis is established when *Brucella* are isolated from the blood or tissues. Trypticase-soy broth and/or Albimi brucella medium are the most desirable

ANTHROPOZOONOSES AND ZOONOSES*

THE "HETEROGENOUS INFECTION" IS INDICATED BY THE COMMON NAME OF THE INFECTIOUS DISEASE, THE CAUSATIVE AGENT AND THE PRINCIPAL ANIMAL RESERVOIRS INVOLVED

A. DISEASES PRINCIPALLY OF DOMESTIC VERTEBRATE ANIMALS

BRUCELLOSIS	BRUCELLA MELITENSIS, BR SUIS AND BR ABORTUS	GOATS, SHEEP, HOGS, CATTLE
TUBERCULOSIS	MYCOBACTERIUM TUBERCULOSIS VAR BOVIS, VAR HOMINIS VAR AVIUM	CATTLE, DOGS, MONKEYS SOME BIRDS
SALMONELLOSIS	SALMONELLA SP	BIRDS, MAMMALS
Q-FEVER	COXIELLA BURNETTI	CATTLE, SHEEP, GOATS, BANDICOOTS
RABIES	NEUROTROPIC VIRUS	DOGS, CATS, WILD CARNIVORES, BATS
LEPTOSPIROSIS	LEPTOSPIRA CANICOLA	DOGS
	LEPTOSPIRA POMONA AND LEPTOSPIRA BOVIS	CATTLE, HOGS
ANTHRAX	BACILLUS ANTHRACIS	MAMMALS
ORNITHOSIS (PSITTACOSIS)	LARGE SIZE VIRUS	MANY SPECIES OF BIRDS (PSITTACINE, PIGEONS, DUCKS, CHICKENS)
ERYSIPELOID	ERYSIPELOTHRIX RHUSIOPATHIAE	SWINE, BIRDS, MICE, FISH
GLANDERS	MALLEOMYCES MALLEI	EQUINE ANIMALS
LISTERELLOSES	LISTERIA MONOCYTOGENES	SHEEP, CATTLE, SWINE, RODENTS
RIFT VALLEY FEVER (ENZOOTIC HEPATITIS OF SHEEP)	VIRUS	SHEEP
LOUPING ILL	VIRUS	SHEEP
COWPOX	VACCINIA VIRUS	CATTLE
CAT SCRATCH DISEASE	VIRUS (BENIGN LYMPHORETICULOSIS)	CATS?
FUNGUS INFECTIONS		
RINGWORM	TRICHOPHYTON SP.	HORSES, CATTLE
	MICROSPORON SP.	DOGS, CATS
RARE INFECTIONS		
MULTOCIDA INFECTIONS	PASTEURELLA MULTOCIDA	RABBITS, RUMINANTS, CATS, BIRDS
PSEUDOTUBERCULOSIS	PASTEURELLA PSEUDOTUBERCULOSIS	RODENTS, BIRDS
MELIOIDOSIS	MALLEOMYCES PSEUDOMALLEI	RATS, GUINEA PIGS, RABBITS, DOGS, HORSES
AUJESZKY'S DISEASE (PSEUDORABIES)	VIRUS	RUMINANTS, SWINE, DOGS
EQUINE INFECTIOUS ANEMIA	VIRUS	EQUINE ANIMALS
FOOT AND MOUTH DISEASE	VIRUS	RUMINANTS, SWINE
NEWCASTLE DISEASE	VIRUS	CHICKENS
OVINE PUSTULAR DERMATITIS	VIRUS	SHEEP
VIBRIO ABORTION	VIBRIO FOETUS	SHEEP, CATTLE
VIRUS B	VIRUS	MONKEYS
HELMINTHIC INFECTIONS		
TRICHINOSIS	TRICHINELLA SPIRALIS	SWINE, WILD CARNIVORES, ARCTIC MAMMALS
FLUKE INFECTIONS	SCHISTOSOMA JAPONICUM, CLONORCHIS SINENSIS, ECHINOSTOMA SP., FASCIOLOPSIS BUSKI, GASTRODISCUS HOMINIS, HETEROPHYES HETEROPHYES, OPISTHORCHIS FELINEUS, PARAGONIMUS WESTERMANII	RUMINANTS, SWINE, DOGS AND CATS SERVE AS RESERVOIR HOSTS
HYDATID DISEASE	ECHINOCOCCUS GRANULOSUS	DOGS, RUMINANTS, SWINE, WILD CARNIVORES
CYSTICERCOSIS	TAENIA SAGINATA AND TAENIA SOLIUM	CATTLE, SWINE
HOOKWORM & RELATED INFECTIONS	ANKYLOSTOMA BRAZILIENSE, STRONGYLOIDES SP., TRICHOSTRONGYLUS SP.	DOGS, CATS AND RUMINANTS SERVE AS RESERVOIR HOSTS
PROTOZOAN INFECTIONS		
TRYPANOSOMIASIS		
AFRICAN SLEEPING SICKNESS	TRYPANOSOMA GAMBIENSE, TRYPANOSOMA RODESIENSE	WILD & DOMESTIC RUMINANTS, WILD GAME
CHAGA'S DISEASE	TRYPANOSOMA CRUZI	CATS, DOGS
LEISHMANIASIS		
ESPUNDIA	LEISHMANIA BRAZILIENSIS	DOGS, CATS, RODENTS
KALA-AZAR	LEISHMANIA DONOVANI	DOGS, CATS, RODENTS
ORIENTAL SORE	LEISHMANIA TROPICA	DOGS, CATS, RODENTS
AMOEBIC DYSENTERY	ENDAMOEBA HISTOLYTICA	DOGS, MONKEYS
BALANTIDIASIS	BALANTIDIUM	MONKEYS, DOMESTIC PIGS
TOXOPLASMOSIS	TOXOPLASMA SP.	MAMMALS? BIRDS

B. DISEASES PRINCIPALLY OF RODENTS AND WILD VERTEBRATE ANIMALS

PASTEURELLOSIS		
PLAGUE	PASTEURELLA PESTIS	WILD RODENTS, RATS
TULAREMIA	BACT. TULARENSE	RODENTS, CARNIVORES, BIRDS
RICKETTSIAL DISEASES		
BOUTONNEUSE FEVER	RICKETTSIA CONORII	DOGS
MURINE TYPHUS	RICKETTSIA TYPHI	RATS, FIELD MICE
ROCKY MT. SPOTTED FEVER	RICKETTSIA RICKETTSII	RODENTS, DOGS
SCRUB TYPHUS	RICKETTSIA TSUTSUGAMUSHI	RODENTS
RICKETTSIAL POX	RICKETTSIA AKARI	MICE
SO. AFRICAN TICK BITE FEVER	RICKETTSIA RICKETTSII VAR PIPERII	DOGS
SPIROCHETAL INFECTIONS		
RELAPSING FEVER	BORRELIA SP.	RODENTS
LEPTOSPIROSIS	LEPTOSPIRA SP.	RATS, MICE
ARTHROPOD-BORNE VIRUS ENCEPHALITIDES	ST. LOUIS, EASTERN AND WESTERN VENEZUELAN EQUINE, JAPANESE B AND RUSSIAN FAR EASTERN VIRUSES	BIRDS, EQUINES, OTHER ANIMALS
SYLVAN YELLOW FEVER	VIRUS	MONKEYS
LYMPHOCYTIC CHORIOMENINGITIS	VIRUS	MICE, DOGS, MONKEYS
RAT BITE FEVER	SPIRILLUM MINUS, STREPTOBACILLUS MONILIFORMIS	RATS

* SELECTIVELY LISTED ROUGHLY ACCORDING TO THEIR PUBLIC HEALTH AND ECONOMIC IMPORTANCE

media. The double medium of Castaneda, in which broth is added to a rectangular bottle and agar is then dispersed along one side of the container has proven valuable (Castaneda, 1947).

(2) Agglutination test. This test, when made with standardized antigens and the tube dilution technique gives reliable results; interpretation must be left to the physician. Unless the patient shows definite evidence of active disease, titers of less than 1:160 must be interpreted with caution. The complicated technique required to reveal blocking antibodies is of little practical value.

(3) Complement fixation test. This may be useful in chronic infection when patients suffering from the residuals of brucellosis may have a low titer of agglutinins. Complement fixing antibodies may persist longer than agglutinins.

(4) Opsonocytophagic test. In the hands of experienced workers this test may yield results of prognostic value. Heparinized blood must be used. The opsonocytophagic test is primarily a research tool, but rarely it helps in the differential diagnosis.

The laboratory methods used for detection of brucellosis in domesticated animals are excellently outlined in the WHO monograph (1953). Detailed instructions are given with regard to (1) examination of fetus and placenta, (2) examination of milk, with special reference to the composition of the dye medium (malachite green and gentian violet) and the modified Ziehl-Neelsen method for microscopic detection of Brucella abortion. In this connection attention must be called to a recent report that tryptose green (Difco Laboratories) with 5 units of penicillin per milliliter of medium and a sufficient amount of gentian violet to give the original dye a final dilution of 1:625,000 suppresses the bacterial flora in cream samples. The use of partially dried plates spread with the specimens and incubated for 5 days in a special incubator in which an atmosphere with 10 per cent carbon dioxide is maintained will in time replace the usual time-consuming guinea pig inoculation method of demonstrating *Brucella* in milk and cream specimens (Gilman et al., 1946). Culture on Albimi brucella agar with polymyxin B sulfate, actidione circulin and crystal violet is an additional useful tool (Kuzdas and Morse, 1953; Hess, 1953). The steps to be followed are described in Chart I, translated from the important paper of Hess and Sackmann (1953).

Brucella abortus, *Br. melitensis* and *Br. suis* are differentiated by their carbon dioxide requirements, inhibition by dyes and hydrogen sulfide production and by serologic tests with monospecific antisera (World Health Organization, 1953a). Dissociation of colonies in the *Brucella* group is detected with the aid of the acriflavin test and the oblique-light technique.

The agglutination test, a highly standardized procedure, has under-

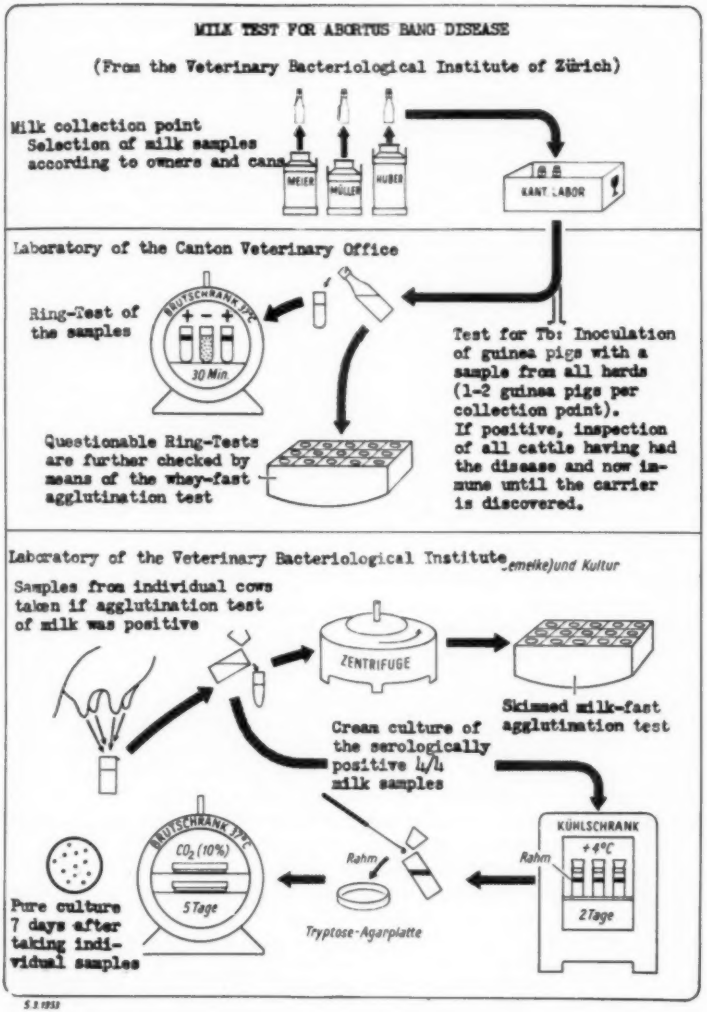
gone great improvements in recent years, particularly the preparation of the agglutination concentrate. This and the *Brucella abortus* agglutination suspension are fully outlined in the section on "Veterinary laboratory methods" in the WHO monograph. Equally instructive are the descriptions of the *Brucella* ring test, which has assumed a prominent position in the rapid detection of infected cattle, in the same section.

Any laboratory work connected with the organisms of the *Brucella* group entails considerable risks to the bacteriologist. Infections with *Br. melitensis* and *Br. suis* are particularly serious. Fortunately recent therapeutic studies give some assurance that the final aim of all treatment of this disease—to protect patients from relapses—may be ultimately achieved. Treatment with both terramycin and streptomycin for 3 weeks and with terramycin alone for 6 weeks thereafter has given the best results (Magill and Killough, 1953).

Anthrax. The incidence of anthrax depends on the level of organization of the livestock industry. All of the eastern Mediterranean area, western and southeast Asia, certain parts of Africa and to a lesser extent Latin American countries, are considered heavily infected. Livestock in these areas is raised or tended predominantly in very small flocks or herds by farmers or nomads whose methods have remained unchanged for centuries. Animals with anthrax left to die in the fields perpetuate and spread the infective agent. Not infrequently the hides, hair or wool are salvaged, sold and frequently reach the world market. Human anthrax is also acquired during handling, skinning or butchering infected animals. In countries in which the livestock industry is well organized, anthrax outbreaks are generally prevented by annual vaccination of livestock. When they do occur they are well controlled, and by-products of anthrax-infected animals are rarely sold. Where the soil is heavily infested with spores, particularly in underdeveloped communities, government agencies must provide low cost or free vaccines of proved potency for livestock, in order to protect the health of agricultural workers.

The relative infrequency of anthrax in the United States rarely brings the medical or veterinary bacteriologist in contact with the problem of diagnosis of this infection. Accidental laboratory infections have occurred in this country, and the bacteriologic principles and techniques taught in every elementary course are rediscovered in tedious trials and errors. Early diagnosis is essential for treatment and prevention of further cases.

Laboratory workers consider that anthrax can be diagnosed easily and quickly by microscopic examination. Experience has shown, however, that it is hazardous to base a diagnosis entirely on examination of smears. Culture and animal inoculation must supplement microscopic examination. Cultures in the transmigration (motility) test substrate and in inactivated horse serum


MILK TEST FOR ABORTUS BANG DISEASE
(From the Veterinary Bacteriological Institute of Zürich)
Milk collection point
Selection of milk samples according to owners and cans
MEIER
MÜLLER
HUBER
KANT. LABOR
Laboratory of the Canton Veterinary Office
Ring-Test of the samples
BRUTSCHRANK 37°C
+ - +
30 Min
Questionable Ring-Tests are further checked by means of the whey-fast agglutination test
Test for Tb: Inoculation of guinea pigs with a sample from all herds (1-2 guinea pigs per collection point). If positive, inspection of all cattle having had the disease and now immune until the carrier is discovered.
Laboratory of the Veterinary Bacteriological Institute
Samples from individual cows taken if agglutination test of milk was positive
ZENTRIFUGE
Skimmed milk-fast agglutination test
Cream culture of the serologically positive 4/4 milk samples
KÜHLSCHRANK
+4°C
2 Tage
Rahm
Rahm
Tryptose-Agarplatte
BRUTSCHRANK 37°C
CO_2 (10%)
5 Tage
Pure culture 7 days after taking individual samples

are more valuable than pathogenicity tests (Norberg, 1953). Over 50 percent of *Bacillus mycoides* are pathogenic for mice, but not guinea pigs. Several strains of *B. cereus* may be pathogenic for guinea pigs, but not rabbits. Both of these organisms are motile; *B. anthracis* is not (Smith et al., 1946).

Rabies. No part of the world is free of the threat of rabies; it is common in the temperate, subtropical and tropical regions and is known to exist in the most northerly Arctic. Along the Mediterranean littoral, in India, Indo-China and the Far East the dog is the most important source of human infections. Eliminating rabies from the dog population is the most challenging problem, since this method is the only one that will protect man against rabies. Traditional control measures frequently are difficult to enforce. Without canine mass vaccination the principal threat to the human population cannot be eradicated. In recent years rabies in wild animals—the fox, the jackal and the wolf—has, in Europe and North America, reached epizootic proportions. The killing of susceptible wildlife to reduce the possibility of the disease being spread from animal to animal and prophylactic preinfection inoculation of livestock and of the dog population exposed to wild reservoir hosts are the methods now employed to protect people living in rural areas. The discovery that vampire bats are infected with rabies in some sections of Mexico and South America has disclosed the existence of symptomless transmitters. The control of cattle and horse rabies by vaccination, although practical on a large scale in regions where vampire bats are prevalent, has not been thoroughly evaluated, nor have the burdens of this measure on rural people been properly appraised.

In an excellent concise summary, Lépine discusses the laboratory diagnostic methods now recommended by the Expert Committee on Rabies of the World Health Organization (1953). Emphasis is placed on the well-known, but too often forgotten, fact that the microscopic examination of smears or impression preparations for Negri bodies should be supplemented by histopathological examination. This recommendation must be followed, in particular by laboratory workers not familiar with the morphology of inclusion bodies and when the result of microscopic examination is doubtful. The fact that the smear examined does not reveal Negri bodies does not rule out the presence of rabies. Sections should be examined for signs of meningo-encephalitis, perivascular cuffing, glia infiltration, formation of encephalitic nodules and ganglion infiltration (lesions of van Gehuchten and Nélis) and different types of neurons should be carefully examined for Negri bodies or lesions of fixed virus rabies. If technical facilities are available, attempts to isolate the virus by inoculation of a laboratory animal should be made. The mouse is the most economical to use, and the incubation time is shortest in

this animal. However, symptoms in the mouse may be atypical and the animal may survive beyond the normal period, although it has characteristic microscopic lesions. All the viruses isolated should be identified by neutralization tests, best executed by a laboratory equipped to conduct such tests and experienced in interpreting the results. (See W. H. O. Monograph 23.)

Salmonellosis. The transmission of *Salmonella* directly from animal reservoirs and indirectly through food products to man is widespread, and the problems it presents are growing throughout the world. Domestic fowl are large reservoirs, and in some regions asymptomatic excreters include cattle, dogs and cats. Despite all precautions the manufacturer, dealer, food handler or food official may take, the ultimate responsibility for preventing single or group outbreaks of salmonella enteritis rests squarely on those who handle food in the public or domestic kitchens and prepare it for the table. Food to be eaten must be fresh, it must be thoroughly cooked in small pieces and cooked long enough to bring the temperature of the entire pieces high enough to destroy the organism. Soft-cooked foods not eaten promptly must be recooked before serving. Human salmonellosis spread through dried eggs can be prevented by cooking the mixtures immediately and thoroughly after the powder has been reconstituted. In countries where these basic measures are not followed the task of suppressing salmonellosis is of appalling magnitude (Meyer, 1953b).

The bacteriology of the genus *Salmonella* is probably more complete than that of almost any other group of bacteria. Biochemical analysis and analysis by means of somatic flagellar antigens have been developed to a high degree of refinement. These advances have rendered the laboratory procedures simple and highly accurate, provided experienced and well-trained technicians follow the analytic procedures. Problems of species identification are referred for final arbitration to a number of regional, national or international *Salmonella* typing centers. The most important details concerning these infections may be found in the review by Hardy and Watt (1950).

Leptospirosis. Until recently human leptospirosis has, with few exceptions in Japan, Australia, Germany and Italy, rarely given rise to dramatic situations that provoke coordinated public health action. As an insidious infection it crept into certain occupational groups—workers in cane fields and rice fields, in coal mines and in sewers. As canicola fever it reaches the dog owner and as swineherd's or milker's grippe it affects agricultural groups. In addition to rats and field mice, dogs, swine, cattle and goats provide suitable reservoirs for this parasite. Now that more reliable diagnostic methods have been developed and inter-

est in the disease has been aroused, leptospirosis has been revealed to be more widely present in domesticated animals than wor formerly believed. Thirty-six serotypes of leptospira have been identified. The recent reports state that bovine, porcine and equine leptospirosis exists in many parts of Europe, Australia, Russia, Palestine and the United States as a severe or mild inapparent infection. Protection of occupational groups constantly exposed to leptospirosis by means of vaccination has been recommended and at least in Japan has proven of value. Control programs to free dairy cattle from leptospirosis by eliminating those with proven serologic reactions are in progress.

Clinical laboratories are being called on more frequently to assist in the detection of leptospiral disease in man and animals. Several monographs describing the procedures for cultural and serologic tests are available (Gsell, 1952; Army Medical Service Graduate School, 1953). They should be consulted and the various pitfalls carefully noted. No effort should be spared to secure cultures on special fluid media, using special peptones, such as Witte's, proteose peptone No. 3 and neopeptone. For blood cultures not more than 3 to 5 drops should be added to 10 to 20 ml. of medium. Contaminated cultures may be injected into guinea pigs by the intraperitoneal route; from 3 to 12 hours after injection the heart blood of the animal is cultured. Thus a pure culture may be obtained. The agglutination test is the most useful diagnostic procedure. Formalinized suspensions can be used for screening tests, but the superiority of living antigens for the differential diagnosis in cases of leptospirosis in man and for the analysis of the antigenic structure of isolated strains is fully recognized. The diagnostic evaluation of positive serologic findings requires considerable experience.

Q fever. Q fever is a world-wide infection. Data on isolation of *Coxiella burneti* from man, animals and ticks, serologically confirmed cases of Q fever and serologic surveys of human and animal populations reflect the existence of this infection in Australia, North America, Africa, Ceylon, India, Japan, Indonesia and highly infected areas in Italy, Germany, Switzerland, Jugoslavia, Greece, Turkey, Spain and Portugal. Since goats, sheep and cattle in these countries are to a varying degree naturally infected and discharge the organism through the placenta and milk, their environment is heavily infected. Dust-laden air of dairy cattle barns and of goat and sheep corrals and pens harboring infected animals continuously endangers the health of man. Since man usually acquires Q fever by inhalation of infected dust, the presence of pulmonary involvement in the clinical disease is not surprising. Early treatment with aureomycin is so effective that chronic infections or complications are rare. Since inhalation of infected dust-laden air likewise maintains

the natural infection among domestic animals, prophylactic vaccination is doubtless the only measure that might ultimately reduce the infection rates and diminish the potentialities of the reservoir.

The laboratory procedures employed in the diagnosis of Q fever are indicated in Chart II, a resumé of the epidemiology of this infection. It is, as a rule, referred to laboratories equipped to conduct suitable tests.

Psittacosis and Ornithosis. The world-wide infection continues to attract sporadic attention in the United States where the predominant reservoir, the parakeet, is responsible for approximately 40 to 50 clinical cases of this disease a year. The annual parakeet population is estimated at 9,400,000. Psittacosis infects psittacine birds and a similar viral agent, that of ornithosis, is found in domestic and ferral pigeons, ducks, turkeys, chickens and undoubtedly other birds. Early treatment with aureomycin or terramycin has greatly reduced the high case fatality rate of nearly 20 per cent that prevailed during the pandemic of 1929 and 1930. Attempts have been made to control importation of infected psittacine birds through quarantine regulations, but they are violated. Control of psittacosis in parakeet aviaries has received attention, but it only temporarily abates the hazard. Reinfection, introduced through trading and bartering with irresponsible bird dealers, breeders and bird fanciers follows, as a rule, and has not been checked without elaborate and unduly expensive administrative apparatus. Educational campaigns have been generally ineffective, because, paradoxically, psittacosis is quite widely considered a myth by the most ardent lovers of cage birds. Prevention of psittacosis is difficult because the nature of the disease is usually recognized only when it becomes epidemic (Meyer, 1952; Meyer and Eddie, 1951, 1953).

The handling of specimens containing the viral agent of psittacosis is accompanied by great risks of infection. It is therefore advisable to refer such material to special laboratories (Meyer and Eddie, 1948, 1954; Meyer, 1954).

It would be profitable to review other public health and diagnostic aspects of the other zoonoses listed in the table. A few are responsible for severe outbreaks or epidemics (plague, jungle yellow fever, arthropod-borne encephalitides), while others are recognized occasionally or in relatively rare instances. Too often it is not appreciated that parasitic infections of animals are just as important sources of disease in man as those incited by bacteria and viruses. For the sake of brief illustration of their importance, three diseases will be mentioned:

Hydatidosis. Hydatid disease, a disease man shares with sheep, cattle and pigs, is extensively distributed throughout the world, but the most important foci are in Iceland, central and southeast

Europe (Greece, Jugoslavia, Cyprus), Turkey and the Arab countries, Siberia, north and south Africa, south Australia, Tasmania and New Zealand, Uruguay, Argentina and Chile. The disease is prevalent only in countries where man, dog and sheep, less commonly cattle and pigs, come into close association. Control measures must be directed towards the prevention of infection in dogs by not allowing them under any circumstances to have access to raw viscera of any of the intermediate hosts (primarily of sheep), by destroying stray and superfluous dogs, by preventing their access to places where they could infect food and to pastures of sheep, cattle and hogs, and by using a powerful taenicide (Arecoline).

Taeniasis. Taenia saginata occurs as an adult tapeworm only in the intestines of man; the larval stage develops in the tissues of cattle where it produces a condition known as "measles" or "measly beef" more commonly than is generally realized. All countries where raw or only partly cooked beef is eaten are infected. Direct personal prophylaxis is achieved if the beef is properly cooked. The grazing of cattle on pastures contaminated with human feces has been stopped in some countries, but this means of control is more difficult where cattle are allowed to roam freely. The problem is one of adhering to the elementary principles of disposal of feces.

Schistosomiasis. The role of animals as sources of this infection needs clarification. Cattle, especially water buffaloes, horses, dogs, cats, rats and in Japan field mice have been found infected with *Schistosoma japonicum*, but their relative importance cannot be estimated with available data. The principal geographic areas—China, Formosa, the Philippines, Celebes and Japan—offer ample opportunities for infection of domestic animals, but their position in maintaining or dispersing the infection has not been determined. If they are proven important, elimination of these sources of infection by systematic treatment deserves serious consideration.

The medical technologist who is called on to assist in the diagnosis of these infections through the examination of feces for helminths and eggs should consult standard reference works (Craig and Faust, 1943; Blacklock, Southwell and Davey, 1953) and seek the advice of experienced parasitologists.

Finally, an estimate of the cost of diseases of livestock and poultry (and the whole story is not told in reported figures) in the United States alone in one year was $1,316,620,000. How far concerted efforts could reduce such figures can be only guessed, but the contribution in terms of health and food should offer an attractive goal to all concerned in the task—medicine, veterinary medicine, livestock owners and pet lovers.


EPIDEMIOLOGY OF Q-FEVER
PNEUMOTROPIC RICKETTSIA INFECTION
RICKETTSIA (COXIELLA) BURNETI
RELATIVELY RESISTANT TO DESICCATION
BROAD HOST RANGE: SUITABLE FOR LABORATORY TESTS-MICE, GUINEA PIGS, HAMSTERS AND EMBRYONATED EGGS
ARTHROPOD INFECTION
ARTHROPOD RESERVOIR
TICKS FECAL PELLETS
TICK-ANIMAL INTERCHANGE
WILD ANIMAL-BANDICOOT & OPOSSUM
BOOPHILUS
AMBLYOMMA
DERMACENTOR
AUSTRALIA
HAEMAPHYSALIS HUMEROSA, CARRIED BY BANDICOOTS AND OPOSSUM
IXODES, HOLOCYCLUS (SCRUB TICKS) CARRIED BY CATTLE, DOG, ETC.
BOOPHILUS ANNULATUS MICROPLUS (CATTLE TICK)
HAEMAPHYSALIS BISPINOSA (CATTLE TICKS)
UNITED STATES
DERMACENTOR ANDERSON (WOOD TICK) DERMACENTOR OCCIDENTALIS
AMBLYOMMA AMERICANIUM
MOROCCO
HYALOMMA
MAMMALIAN RESERVOIRS IN EUROPE AND OTHER COUNTRIES
INHALATION, INGESTION
DUST FROM STRAW AND WOOD PACKING
PLACENTA (MILK)
PLACENTA MILK MEAT & BLOOD
AIR CONTAMINATION
INGESTION
PATIENT 1 NURSE, 2 PATHOLOGISTS; AUTOPSY ROOM
CARRIER 36 PATIENTS AND STAFF
AUTOPSY 18 STUDENTS AND PHYSICIAN
DIAGNOSTIC PROCEDURE
1. SEROLOGIC TESTS (HIGHLY SATISFACTORY AND RECOMMENDED) AGGLUTINATION (AGGLUTININS RARELY APPEAR BEFORE THE 9th DAY USUALLY 4 WEEKS) COMPLEMENT FIXATION AFTER 7 DAYS
2. ISOLATION OF RICKETTSIA BURNETI (ONLY IN EQUIPPED LABORATORIES) FROM BLOOD, SPUTUM, SPINAL FLUID, URINE, OR TISSUE OBTAINED AT AUTOPSY, BY INOCULATION GUINEA PIGS, MICE, HAMSTERS, MONKEYS AND EMBRYONATED EGGS
3. ALLERGIC TESTS
PROTECTION OF LABORATORY WORKERS BY VACCINATION

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THE GAVEL

Irrevocably and steadily our profession is being challenged by a new responsibility. There is an increasing demand for our active participation in teaching and training programs. Since this duty is becoming an important aspect of our professional lives, it should be given serious thought. What is going to be demanded of us today to fulfill our obligations to the young men and women who will be the Medical Technologists of the future?

First of all is enthusiasm, a real devotion and deep love of our work, without which instruction becomes routine and dull. Second is knowledge, wide and varied personal experience as well as energetic, continuous study, without which classes are meaningless. Third is a sense of proportion or judgment, an inherent natural balance of animation and conscience, without which the teacher cannot reach the student. Fourth, a keen sense of consideration and obligation, a love of people that impels one to contribute to the welfare of people, without which that indefinable spirit of devotion to duty and high ideals is lost to the student.

How will each of us respond? What will this mean to each one? It will be exactly as the individual himself wants to make it. For some it will be a worry, a care, an annoyance. For others it will be a life of useful accomplishment in watching the students, with humility, grow in confidence, knowledge, and hope.

As a medical technologist it is with tremendous pride in my profession that I believe this new phase of our growth is becoming a source of inspiration for many of us and that we are facing the challenge with energy and integrity.

Ruth Hovde

EDITORIAL

You are a recruiter! Whether or not you are actively interested in recruitment, *you are a recruiter*. According to the survey conducted last Spring by the ASMT Vocational Guidance and Recruitment Committee among students in Approved Schools, those students became interested in the profession primarily through contacts with Medical Technologists.

You are a symbol of your profession, each day, to all whom you meet; on the job, at home, or anywhere you may go. You have a duty to yourself and to your profession to represent Medical Technology well. Keep informed. Know the pre-technical college requirements and the names of the Approved Schools in your area. Show interest and enthusiasm about your profession. Talk shop.

Do you remember the reasons why you entered the field? Do you sometimes in the press of daily work lose sight of your primary motives and do accurate work merely because of pride in perfect technique, work accomplishment, or loyalty to your employer? These are worthy objectives, but if they are all you have, with the passage of time, they will not be enough, and your work, instead of inspiring, will become routine and uninteresting. The students recently surveyed indicated that their main reasons for entering Medical Technology were: an interest in science, a desire to work with human beings, and a career that would serve humanity. The last two combined were the impelling incentives to 80% of those answering. Weren't these your reasons, too? Do you stress those facets of your profession when talking about it? Or, do you get so involved in apologizing for the salary, the night calls and Sunday work, that you forget the motivating force behind it all? Would you do this work for just the salary if there were no human beings involved? Can you measure your work with the secretions and excretions, the unpleasant odors, the hand-staining dyes, the burning chemicals, and the eye-fatiguing counts in

dollars and cents? No, I don't think you can. So, why think potential recruits would? Youth is very idealistic—ready to die for a cause or to live for humanity. The display of our armed forces in the last wars is proof of this.

If you are to sell the youth who have an *interest in science*—your profession, you must stress your talking points: *service to humanity*. You must impress them with the fact that that service will be visible to them, easily evidenced daily in the results of their tests.

If you sort out those interested in science and stress the humanitarian aspect to them, you have the two keys to success in securing recruits. All other allied fields in the medical profession can also point out this service aspect, but no other one really requires a sound interest in pure science. Those individuals who have that attraction to science need to be awakened to the existence of the profession of Medical Technology and its profound necessity in securing community health.

So more programs should be initiated to propagandize the profession in all ways feasible, but they must be a superimposition on personal contact. You should always bear in mind that as a Medical Technologist, YOU are the best salesman for your own profession. If you are a good Medical Technologist, you can be a good recruiter.

Why should *you* be a recruiter? If you love your profession, if you have found happiness in serving others and you want to share that pleasure, *you* should be a recruiter. Since you don't want the enthusiastic youth to be channeled into routine and uninspiring occupations, *you* should be a recruiter. Because you wish them to have the same soul-satisfying work as you do, to share in your life-saving science, *you* should be a recruiter. Because you wish only well-trained, enthusiastic individuals working with you, Technologists acutely aware of the service to mankind inherent in their work and properly imbued with the ethics of the profession, *you* ARE a recruiter.

Integrity, self-sacrifice, and service, are the symbols of your vocation; carry them high and proudly and many will follow in your wake.

Audrey Murphy

MEDICAL TECHNOLOGIST TRAINING IN PROTHROMBIN TIME DETERMINATIONS

By ANNA FAGELSON, B.S., M.T. (ASCP)[1]

More than half of all deaths in the United States are caused by cardio-vascular-renal disease, and many of these deaths are due to thrombosis and embolism. While hardly more than a start has been made toward the conquest of the cardiovascular diseases, one of the major advances in their treatment is the discovery of anticoagulant drugs. Discoveries which mark the advance of medical science, however, very often bring with them totally new problems. The restoration of health and the prospect of continuing usefulness for the patient may depend on the accuracy of laboratory procedures. This is especially true in the use of anticoagulants. Too large a dose may lead to hemorrhagic complications while too small a dose may be ineffective. The prothrombin and blood clotting determinations are crucial in

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helping the physician use anticoagulant therapy effectively. Thus the medical technologist plays a vital role and must be skilled in these procedures.

Unfortunately, there are still some who believe it is not necessary for the medical technologist to be concerned with anything but the mechanics of laboratory procedures and therefore can be adequately trained in a few months. The registered medical technologist has found that even the required minimum training period of twelve months can provide little more than a foundation upon which he must build when and if the opportunities arise.

The mechanics of the prothrombin test are not difficult, but there is much more to this test than mixing thromboplastin, plasma, and calcium chloride. Because of changing concepts, the complexity of terminology, and the multiplicity of procedures, it is not an easy task to perform the most reliable prothrombin determination. Very often hospitals and laboratories in the same community lack standarized procedures and may not have uniform reporting methods. Of course, this handicaps the clinicians in interpreting the results of such tests.

In order to alleviate confusion concerning anticoagulant therapy and control, several State health departments have provided refresher training courses in prothrombin and blood-clotting determinations. In making arrangements for these courses, some of the health departments have obtained the assistance of the Public Health Service, U. S. Department of Health, Education and Welfare, in the form of consultation and help in planning and conducting the training programs, and, where necessary, have used the services of a specially trained, full-time instructor provided by the Public Health Service.

In planning these courses, attention has been given to the possibility of meeting the long-term training needs of medical technologists through training one or two technologists in a laboratory supervised by a clinical pathologist. These technologist-instructors can subsequently teach refresher courses in various parts of the State.

One of the objectives of the prothrombin refresher course offered by the States in cooperation with the Public Health Service has been to strive for the standardization of procedures. Methods for reporting the results of these procedures and methods for eliminating common errors have been especially emphasized. These refresher courses have given the medical technologist the opportunity to gain familiarity with the fundamentals of blood coagulation and to explore and solve problems of procedure and technique, thus gaining valuable knowledge and experience which he can effectively utilize in his laboratory work.

THE WORK OF THE REGISTRY OF MEDICAL TECHNOLOGISTS

LALL G. MONTGOMERY, M. D.

Chairman, Board of Registry, Muncie, Indiana

It is a great disappointment to me not to be able to attend the meeting of the American Society of Medical Technologists this year, because it is always so pleasant to renew old acquaintances and make new ones. Then, too, there is nothing like talking with you folks and listening to your excellent programs to give me a sense of direction which is so helpful in the year that follows. I am grateful that Doctor Squires has been able to be here as a representative from the Board of Registry, and I am sure that she and Mrs. Drummond will give generously of their time and advice when needed. I will look forward to hearing their report of your meeting.

You may remember that last year I talked about what had happened in the first twenty-five years of the Registry's life, and also mentioned some of the things that were projected for the future. This past year has been a year of new plans, of further accomplishments, problems both old and new, and an ever-increasing amount of work to be done. There have been times when we felt like the Red Queen in Lewis Carroll's "Through the Looking-Glass," when she said ". . . it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!" Well, we couldn't stay in the same place, so we have just had to run twice as fast. We have been encouraged to see many of you along the way, going at the same pace, and I know that you have enjoyed it as much as we have.

Total Registration

One of the principal reasons for this accelerated pace is a purely statistical one—the yearly growth in the number of registrants brings more correspondence, more renewals, more changes of address to take care of, more cards and seals and TECHNICAL BULLETINS to be mailed.

The total number of medical technologists registered since 1928, including those registered as a result of the 1954 Spring examination, is 25,306, of which 19,969 are currently registered. During the past year 533 medical technologists have been withdrawn from the roster, including 459 for delinquent fees and/or lost address, 42 resignations, 19 deceased, and 10 withdrawn for violations of the Registry Code of Ethics. There were 57 former registrants re-instated during the year.

In addition to the medical technologists, there are now 5 registered as specialists; 30 microbiologists; 29 chemists; 230 his-

tologic technicians; and 69 laboratory aides. As you will recall, we have not certified anyone in the latter category for the past three years, as such certification is being held in abeyance pending a definite decision of the American Society of Clinical Pathologists regarding continuance of this category.

We thought it would be interesting to keep a record of the number of address changes that were made during the rush renewal season. For the months of December through April, there were 3,871 changes of address made, from correspondence from registrants and from renewals. This is an average of 774 a month for those five months. In one month, 1,393 changes were made; 1,032 were from renewals and 361 from correspondence. As you can well imagine, the renewal department is especially busy during December and the first few months of the year.

Registry Mail

The incoming mail count is always a barometer which indicates the amount of work to be done in the Registry office, and the outgoing mail count an accurate indication of what has been done.

During the past year we received a total of 49,430 pieces of mail, an increase of 5,294 more than for the same period for the previous year. The largest increases have occurred in the number of renewals (which is to be expected), the number of orders (largely due to the publication of the third edition of A CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY), correspondence regarding applications, changes of address and mail returned because of such changes, requests for guidance material, and miscellaneous mail, which includes questionnaires returned by themselves—that is, not with renewals, with which they were mailed out.

During the same period, a total of 71,539 pieces of first class mail went out from the Registry office. Records are kept of the breakdown into various categories of mail, and also how much each of the office staff members handles. In this way it is easy to check on the flow of work in the office, and to decide where additional help is needed during peak periods.

I mentioned that one of the categories of incoming mail that shows an increase is that of the requests for guidance material. Of course this also shows up in the outgoing mail count. An increase in recruitment activities has been reflected in the total figure of 161,507, which is the number of pieces of material which was sent out in large quantity shipments. It went to such places as your national office, your state and local recruitment committees, to career conferences, state fairs, Health Fairs, for National Laboratory Open House or National Hospital Day activities, to national organizations which were including material about the profession of medical technology in their regular

mailings to their memberships. This year's figures of 161,507 pieces represents an increase of 42,992 over the amount sent out last year. In addition, selected pamphlets were sent to 2,275 who requested general information about certification or about medical technology as a profession, and marked booklets and/or lists of Approved Schools were sent to 1,192 who asked specific questions which could be answered by marking certain sections in the booklet or school lists. Incidentally, you will be interested to know that the postage and express charges for shipping the material that was sent in quantity mailings amounted to $513.70 for the year. The actual cost of the 161,507 pieces of material mailed out was $4,900.11. This is a graphic demonstration of the amount of work you folks have done in the way of recruiting and publicizing the profession, because the greatest part of the material went to you for use in your local, state and national recruitment activities.

Approved Schools of Medical Technology

The increase in the number of Approved Schools of Medical Technology increases the amount of work to be done in the Registry office, although the great burden of the work in connection with the actual approval is done by the Council on Medical Education and Hospitals of the American Medical Association and the Board of Schools of Medical Technology of the American Society of Clinical Pathologists. But as new schools are added, our correspondence with prospective students and with the pathologists and other instructors in the schools necessarily increases.

There are now as of February 1954, 576 Approved Schools, with a total student capacity of 4,275. No doubt your representatives on the Board of Schools will give you a report at this meeting, and will tell you that many of the schools still do not have enough students to fill their classes to capacity. However, it is encouraging to hear that there is an increase in the number of schools which have more applicants than they can accommodate for the coming year.

During the past year the Board of Schools of Medical Technology and the Council on Medical Education and Hospitals of the American Medical Association made some revisions in the official statement of the "Essentials of an Acceptable School of Medical Technology." This revision appeared in the November 7, 1953, issue of the Journal of the American Medical Association, and has been reprinted in the last two revisions of the Registry booklet. You will want to be sure to read it and to note the changes that have been made.

One of the Registry's important responsibilities toward the Approved Schools is to make evaluations of the college credits

of the prospective students. During the past year we have made 3,017 transcript evaluations, including original evaluations, revisions and copies. We have been interested to note that during the first four months of this year we have made a total of 884 original evaluations, as compared with 733 for the same four months last year. This could mean one or more of several things; there are more Approved Schools, and therefore more students whose transcripts are to be evaluated, more students of the right age group are becoming available for training, consistent recruitment is bearing fruit and more students are considering medical technology as their chosen work. Probably it is a combination of all of these factors, but at least it is an encouraging trend.

Registry Examinations

The Board of Registry accomplished something this past year which we believe will do much to aid the Approved Schools in improving their teaching programs. You who are teaching are familiar with the method we now use to report to the Approved Schools the results of the examinations. The record of the student is given in each section of the examination, the passing mark is given, and the percentile rank for each student. Thus the pathologist and his assistants can easily determine where the teaching program is adequate, or where it needs to be improved. Those of you who have had experience with testing know that the reports we send out are but a very small part of the whole process of scoring, recording and analyzing an examination. An enormous amount of time and money has gone into preparing this new material for the Approved Schools, but we believe it will be well worth the effort and expense. The credit for carrying out the Board's instructions regarding this phase of its work goes in large measure to Doctor Arbogast, who has been tireless in his work on the new methods of analyzing and reporting the examination results. He is preparing an article about this work, which we hope will be published soon as a further aid to you who are teaching in the Approved Schools.

Certification of Blood Bank Technologists

At the request of the American Association of Blood Banks, and with their complete cooperation, the Board of Registry is planning to certify Blood Bank Technologists, the first examination to be given next fall. The possibility and feasibility of certifying Blood Bank Technicians was discussed by the American Association of Blood Banks quite thoroughly and for some time before they requested the Board of Registry to consider the problem. In keeping with its long-time policy of considering requests from such special groups when it is apparent there is a

need for a new classification, the Board of Registry was glad to give careful consideration to this request.

Definite commitments were made at the October 1953 Board meeting. Subsequently, the Education Committee of the American Association of Blood Banks has met several times to discuss training of Blood Bank Technologists, curriculum, qualifications for certification, examinations, etc. Two members of the Board of Registry serve on the Education Committee of the American Association of Blook Banks, Doctor J. L. Arbogast and Doctor Gretchen Squires. As is stated in the most recent edition of the Registry information booklet, candidates for certification in this field must be medical technologists registered by the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists, and must have had three months of approved training in Blood Banking, plus nine months of practical experience subsequent to training. Persons who were trained previous to January 1, 1954, may substitute one year of current experience in Blood Banking. Both a written and a practical examination will be given. For information about the courses and a list of the Blood Banking Laboratories approved for this training, candidates should write to Miss Marjorie Saunders, Secretary of the American Association of Blood Banks, 3500 Gaston Avenue, Dallas, Texas.

Technical Bulletin

Beginning with the January issue of this year, the TECHNICAL BULLETIN has been mailed from Baltimore, Maryland, where it is printed. This change in the mailing was made in order to give you better service, and we believe that it has been a good move, as there is now no lag between publication date and the date of mailing, as there was when the BULLETINS were shipped from Baltimore to Muncie for mailing. Several months ago we mentioned that the cost involved in re-mailing the TECHNICAL BULLETIN had increased, and we appealed to you to send in changes of address promptly to avoid delays and additional mailing costs. We guarantee return postage, which is four cents for each copy returned to us. Over the past year it has cost us $90.17, just to get the undeliverable copies back. Then we send double post cards, costing four cents, to get a confirmation of the address given to us by the post office. When you return the card, confirming the address, we pay an additional three cents to re-mail your BULLETIN. In other words, it costs us eleven cents *in addition to the original mailing cost,* to re-mail a BULLETIN undeliverable because of a change of address which was not drawn to our attention by a registrant, in time to be changed for that month's mailing.

"A Curriculum for Schools of Medical Technology"

As announced last year, the third edition of A CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY by Doctor Israel Davidsohn and six co-authors was published last June. A total of 1730 copies has been sold to date, and as new classes enter the training schools, we expect this excellent record to continue. I am sure that you who are teaching in the Approved Schools are finding it useful, and no doubt you are suggesting to your students that it is an invaluable part of a medical technologist's library after she is through school, as well as a guide to study while still in training. I hope that you have all discovered the "Introduction," with its many splendid suggestions about the training of medical technologists.

As has been announced to you before, both Doctor Davidsohn and I turned over to your Society the payments we received for our part in preparing the CURRICULUM. I am sure that you will hear more about how this money is going to be used.

Registry Exhibit

Three sets of the Registry exhibit have been used at nineteen meetings during the year. They have been used at your national meeting, and several state meetings, at state and county fairs, at a Health Fair, at the annual meeting of the American Personnel and Guidance Association, at the American Society of Clinical Pathologists meeting, and the spring meeting of the International Association of Medical Museums and the American Association of Pathologists and Bacteriologists, at the annual convention of the American Medical Association, state medical society meetings, and one state Hospital Association meeting. Many of you have tended the booths where the exhibit was used and have given us valuable suggestions about its use. If you have not already done so, we would like to have you see our new exhibit, used for the first time at this meeting. We hope that you will agree that it should attract attention to the profession of medical technology and to the length of time necessary to get the education and training required for certification.

Educational Fund

During the past year the Educational Fund sponsored by the Board of Registry has continued to be administered by the Education Committee of your Society. Applications for grants from the Educational Fund provided by the Board are submitted to your committee, which evaluates the requests and accepts or rejects them, according to whether they meet the requirements established for eligibility for use of the fund. The Board of Registry then sends checks to the societies whose applications have been approved by your Education Committee. I am sure

that the chairman of that committee will give you a report at this meeting, and probably will tell you how many applications from state medical technology societies received their approval. I would like to commend your committee for its work in administering the fund during the past year. It has been interesting to see the kind of seminars that have been presented, and to know that this fund is serving well the purpose for which it was established several years ago.

Questionnaire

We have talked about what has happened during the past year, and have tried to evaluate it in the light of our present problems and future plans. I think that one of the most valuable yardsticks for evaluating the present and estimating our future needs is the questionnaire which was sent to all registrants with the 1954 renewal blanks. I must confess that we sent it out with a few misgivings, because we simply could not cut it down to a length which seemed reasonable. We were afraid that the great length and the complexity of the questions might discourage you, but we have been astounded and delighted that the results have so far exceeded our actual expectations. We mailed 17,710 questionnaires with the renewal blanks. Up to the first of June we had received 10,486 which is 59 per cent of those sent out and an excellent return statistically. We have received 15,201 renewals, so 72.4 per cent of those renewing have returned their questionnaires.

As we told you in our annual letter, the information on the questionnaire will be put on punch cards. But a great deal of work must be done on the questionnaires in the Registry office before they can be turned over to the punch card operators. For instance, we have to see that all answers are in the proper places on the forms, that quarter or term hours are converted to semester hours, we have to give a key number to the state in which the registrant works, and a number to the school where she had her technical training. If information given on one part of an answer contradicts that given in another part of the answer, we must mark that question so that that particular questionnaire can be followed up with a letter to the registrant, to get the correct information. So each questionnaire must be gone over carefully, and this is why we do not expect to have the analysis ready until late in the summer. When the punch cards are completed, we will be able to "ask the machine" to tell us certain things, and it will promptly give us the answers to questions which have often been asked before but which we have never been able to answer. Of course this will be statistical material only, which will then be sorted out with the hope that

it will at least point the way to answers to some of our most urgent problems.

Your Standards and Studies Committee has given us excellent advice and suggestions all the way along. They suggested several questions to be included, and are working on suggestions as to what we want to find out from the analysis. They have gone over the large stack of answers to the "64-dollar" question, (Question No. 63, which asks what you would look for if you were choosing a pathologist to work for), and perhaps will have a report for you later at this meeting. Several articles could be written around the answers to that question alone, to say nothing of the various other elements of the questionnaire. We hope to have the most valid information we have ever had on salaries; about your ideas on educational requirements, recruitment; about conditions of employment; place and type of employment; stability of employment. On behalf of the Board of Registry, I want to thank you for your help as individuals and as members of committees, and to assure you that we will complete the project as quickly and with as much careful appraisal as we possibly can.

Recruitment

For several months you have been hearing about the National Committee for Careers in Medical Technology. As you know, this committee is composed of representatives from the American Society of Medical Technologists, the American Society of Clinical Pathologists and the College of American Pathologists, with consultants from the Board of Registry and the Board of Schools of Medical Technology, as well as several expert consultants from outside sources. No doubt Miss Hovde will give you a full report of the activities of the committee, because as you know she is your representative on this committee. She will also tell you more about the film which will have its Premiere Showing at the meeting of the International Congress of Clinical Pathology in Washington in September. Later on you will learn how you can collaborate with the committee in distribution of the film in your own communities.

We have stressed recruitment at every meeting for several years, and it remains the most important element of your work and ours. Your members have worked long and hard on the recruitment program, contributing time and ideas and money. The present plans, coordinating your work with the work of the American Society of Clinical Pathologists, the College of American Pathologists and the National Committee for Careers in Medical Technology, should do much to further the program and produce the desired results.

The Challenge of the Future

With the means to evaluate the present (the analysis of the questionnaire), and the tools to help us mold the future (the new film and other recruitment aids), there is no reason why we cannot say with complete honesty and confidence that the future of laboratory medicine can be assured. I say "can be," because this depends on the way we meet this challenge—the way we meet it as individuals, the way we meet it as members of our professional societies. Our progress toward the goal calls for a united front, for the ability and willingness to put aside petty differences, for the objective attitude that we as mature professional people should have attained. Let us remember, above everything else, that we have *chosen* this profession, not only as a means of livelihood, but because we want to do our part in caring for the sick. The patient and his welfare must be paramount in our every thought and plan and action. Deviation from this attitude could be disastrous to your own profession and to the ideals which have been developed for it.

It is with a great feeling of increased hope and confidence that I bring you this brief account of the past year and join you in looking forward to a fine year ahead.

WHAT REGISTRATION MEANS TO ME*

By Sister M. Coronata, M.T. (ASCP)

(Sister M. Coronata is chief technologist at St. Joseph's Hospital in Minot, North Dakota. She had her laboratory training at Mercy Hospital in Denver, Colorado.)

As I write these lines, I can look up on the wall of the laboratory to a certificate in a simple frame—a small certificate with a row of cheerful red seals across it face, which seems like the face of an old friend. Coming fresh from school to this hospital to replace a Sister who had been more than fifteen years in the laboratory, I felt very "green" and inadequate, and the dubious glances of some of the older doctors were not reassuring. It was a happy day, therefore, when I received that certificate from the Registry and I made haste to hang it in plain view. Now everyone could see that I was prepared for my new work—the ASCP said so!

Through the busy years since then, that bit of paper and what it represents has always been a morale builder. Hospital inspectors and new staff members have nodded approval on seeing it. When it came to hiring additional help in the laboratory, no one ever brought in a better recommendation than the little green card from the Registry.

Much is made of the nurse's cap as "the symbol of a proud profession." To my mind, the seal of the Registry is no less so. With the increasing use of laboratory aid in medicine, our work seems destined to even greater significance in the future. May the Registry of Medical Technologists ever continue to lead us on and to keep our standards high!

* From the News Bulletin of the North Dakota Society of Medical Technologists, April, 1954.

THE RELATIONSHIP OF BACTERIAL RESISTANCE TO SOME ANTIBIOTICS

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Many changes have occurred in the field of microbiology since the advent of penicillin and similar antibiotics and chemotherapeutic agents some few years ago. Many problems, too, have been presented with the concept of antibiotics. For instance, the challenge of discovering new antibiotics which would vary in antimicrobial powers, their chemical properties, and the toxic effects produced. Evidence has been presented which shows that antibiotics and chemotherapeutic agents may produce an undesirable effect upon certain bacteria. These can be: acquired resistance, dependence or stimulation.

It seems that the least familiar of these effects is stimulation. Stimulation is produced by concentrations lower than those required to inhibit growth; in the case of naturally resistant organisms, this concentration may be among the highest attainable in the body. One of the most important, possibly even *the* most important, is that of the developing of resistance by microorganisms to certain antibiotics.

These problems have ever increased in dimension as interest has increased in the field of antibiotics along with the discovery and production of a tremendous number of chemotherapeutic and anti-infective agents. A general belief at present is that development of resistance of bacteria to antibacterial agents such as antibiotics, arises spontaneously as a genetic mutation among a few cells. Thus the non-resistant ones die, leaving the resistant ones to multiply. Perhaps the mechanism is based upon the ability of the antibiotics to interfere with one or more of the metabolic processes of the organism, preventing the utilization and absorption of nutrient materials or the excretion of waste materials. Perhaps too, the bacterial cell varies in its degree of resistance during each stage of its life cycle. Thus in any given agglomeration of bacteria, most or all stages of the life cycle are represented. Some bacteria are capable of developing new or perhaps utilizing other existent enzymic reactions and thereby continue to live. The indiscriminate use of drugs and self treatment which bring on resistance, should also be taken into consideration in the overall picture of such development.

Two patterns for the development of in vitro microbial resistance to antibiotics are commonly recognized. Resistance develops in one pattern (characteristic of penicillin) only in a slow, step-wise manner during serial transfer in progressive concentrations of the antibiotic. A "step-wise" development is also

characteristic of the streptomycin pattern but they differ significantly in the manner in which high-level resistance is attained. With penicillin, the first step mutants are very uniform in their degree of resistance, which is only slightly higher than that of the original strain. Additional mutations occurring in such first-step mutants result in bacteria possessing a higher (second-step) degree of resistance, and in a similar manner third-step and so on higher resistance develops. Always the variation in degree of resistance among mutants of the same step is slight. This uniformity constitutes the most striking feature of the penicillin pattern. The streptomycin pattern of resistance differs from the penicillin pattern in that the variation between first-step mutants is very great. They range from individuals only slightly more resistant than the original bacteria to those having complete resistance to streptomycin. Therefore, resistance may not only be attained in successive steps, as with penicillin, but may also arise in a first-step mutant. Should a microorganism develop resistance to an antibiotic with relative ease it is evident that the therapeutic value of said agent may be seriously limited. Perhaps the most widely known and talked of microorganism which has demonstrated a particular affinity for resistance developing behavior to antibiotics in general and that of penicillin in particular is the staphylococci. Garrod[1] has summarized the possible effects of these chemotherapeutic agents on bacteria as follows: *first*, they may be inhibitory or lethal: *second*, the microorganism may acquire resistance; *third*, dependence upon the drug may develop, and *lastly*, the effect may be actual stimulation of bacterial growth. In this paper we are chiefly concerned with the second category, that of the ability of bacteria to acquire antibiotic resistance.

It seems that staphylococci possess two kinds of resistance to penicillin.

(1) One is that which can be induced artificially *in vitro*. (First described in 1941 by Abraham, et al.[2]) This type of resistance appears to be temporary in duration, with a fairly rapid return to almost the previous state of sensitivity following transfers in penicillin-free medium.[3] Klimek, et al.,[4] in a study of developing resistance of staphylococci to both penicillin and streptomycin, noted that morphological and gram-staining changes occurred, the cocci becoming pleomorphic, rod-shaped and gram-negative.

Resistant variants may lose their ability to grow anaerobically, to ferment the usual sugars, with the exception of glucose, and to grow in 6.5% sodium chloride.[5] Segalove and Hite,[6] in examining the effect of penicillin on toxin formation of both types of penicillin-resistant staphylococci found that in particular culture media, production of rabbit and sheep red blood cell hemolysins, lethal factor and dermonecrotoxin was inhibited by peni-

cillin, while there was not any inhibition of enterotoxin formation. Preformed staphylococcal alpha hemolysin or lethal factor was not neutralized by the addition of penicillin. While these various factors were being investigated the realization that another kind of staphyloccal resistance to penicillin existed came into focus through clinical observations.

(2) This being that some strains of staphylococci were insensitive to penicillin upon primary isolation. Kirby[7] first demonstrated the presence of an inactivator in extracts of seven strains of penicillin-resistant staphylococci and its absence from seven penicillin-sensitive strains. Bondi and Dietz[8] used the term penicillinase to describe this inactivator. The work of Gilson and Parker[9] showed that the amount of penicillinase produced by naturally resistant staphylococci may vary by a thousandfold. Resistance of an infection to treatment with penicillin may therefore be expected to vary according to the amount and rate of production of penicillinase by the infecting strain. At present it is believed there is a possibility that increased resistance to penicillin can be the result of two entirely different mechanisms. One of these is the development of an ability to produce penicillinase, and the other, that strains of staphylococci believed to have acquired resistance *in vivo* may really have arisen from naturally insensitive cells.

Another view, championed by Abraham, et al.[10] was that development of increased resistance to penicillin is a direct result of exposure to the antibiotic and is an adaptive change both inheritable and permanent. Demerec[11] has suggested that antibiotic-resistant cells occur as rare spontaneous mutations in the course of bacterial multiplication and that they are present in the original colony, with the antibiotic exerting a selective action. It is presumed that, ordinarily, these first-step mutants are overwhelmed in numbers by the penicillin-sensitive cells, but, during exposure to suitable concentrations of penicillin, these are inactivated or destroyed, leaving the resistant individuals to grow out. Eagle's et al. [12] observations indicate that the number of bacterial cells capable of growing out to form colonies declines with the concentration of antibiotic. The resistant colonies and their progeny are regularly more resistant than the original one, and, in general, this resistance is related to the concentration in which the resistant colony emerged. For example, at low antibiotic levels, 10% to 90% of the bacterial population grew out to form slightly more resistant individuals, while at higher levels, only a few appeared. There was no indication of a step-wise emergence, but rather an even falling off between low and high concentrations.

Therefore, with these postulates, we may place the possibilities for development of increased resistance into two categories. One, that at low level concentrations, a large percentage of bac-

terial cells will grow out to form colonies of individuals which are slightly more resistant than the parent cells. These may be either rare spontaneous mutants appearing during bacterial multiplication in the presence of the antibiotic and overgrowing the sensitive bacterial cells, or strains which develop an adaptive process in the presence of the antibiotic. *Two,* that at high or substerilizing concentrations of the drug only rare individual cells grow out to form highly resistant colonies. These are considered to be either "spontaneous mutants present in the original inoculum, and which grow out selectively in the presence of the antibiotic" or "the result of an adaptive process in the presence of the drug." There is considerable evidence that antibiotic-resistant staphylococci is appearing with increasing frequency in many hospitals; more so than in the community at large. This is especially true of English hospitals where penicillin was first developed and could partly be the result of the transfer of resistant strains to hospitalized patients from other patients and from the attendants. According to Rountree, et al.[13] the carrier rate of resistant organisms is higher among hospital personnel than among the general public, with the incidence of Aureomycin-resistant strains increasing steadily. The hospital environment permits the spread of resistant variants to large numbers of people. The bacterial air population in hospital areas contains more antibiotic resistant organisms than non-hospital air.[14] We see from these investigations that the high environmental contamination of hospitals with resistant staphylococci results in a risk to the patient of acquiring such infection, since he is exposed during a time of lowered resistance.

As the antibiotic-resistance of staphylococci has been discussed at length let us briefly discuss microbial resistance to a few other chemotherapeutic and systemic anti-infective agents.

Aureomycin

It has been shown in vitro to be capable of attacking both gram-positive and gram-negative bacteria as well as certain large viruses and rickettsia. From experimental data it is said that tolerance to Aureomycin may be induced by the serial subculture technique fairly readily in most strains of gram-negative bacilli; less readily in some strains of staphylococci, and with considerable difficulty in streptococci, especially of the beta-hemolytic type.[15] Resistance is rarely increased more than two, four or eightfold, and it is of significance that acquired resistance tends to disappear promptly after exposure to the antibiotic has ceased.[16]

Critical reviews of the literature on Aureomycin in 1950 stated that the development of resistance has not been a clinical problem.[17-18] The same consensus of opinion has held through 1951[19] and 1952.[20, 21, 22]

Magnamycin

Magnamycin is principally active against gram-positive bacteria with little or no activity against gram-negative types. In addition, magnamycin possesses effective inhibitory activity against rickettsia and the large viruses. Preliminary studies demonstrate that test organisms acquire resistance to Magnamycin in a very slow, step-wise fashion when transferred serially in the presence of antibiotics. A significant factor of Magnamycin is that it does not appear to possess cross-resistance with other antibiotics such as Penicillin, Streptomycin, Terramycin, Chloramphenicol, Aureomycin, Polymyxin B, and Bacitracin. Of particular interest is the fact that some infections caused by streptococci and staphylococci resistant to other antibiotics have responded to this drug. Data indicates that the resistance pattern of Magnamycin is comparable to that of Penicillin,[23] Terramycin,[24] etc. and is in contrast to that of streptomycin. If cross-resistance does occur between Magnamycin and any of the seven commercially available antibiotics, it does not occur to a high degree.

Chloramphenicol

Chloramphenicol (Chloromycetin) was one of the first important antibiotics produced synthetically. It is of value not only in many gram-positive and certain gram-negative infections but also in a number of diseases caused by rickettsiae, spirocheates and viruses. At present it is *the* drug of choice in the treatment of typhoid fever.

Gocke, Finland and Wilcox conducted studies with Aureomycin, Terramycin, Chloramphenicol, and Neomycin using 14 bacterial strains. About 40 serial transfers were made on solid media containing progressively increasing concentrations of these antibiotics. From these studies they reported that resistance to the homologous antibiotic showed the most regular and rapid increase in the case of Neomycin; this also occurred regularly, but not so strikingly, with terramycin. Only some of the strains became increasingly resistant to aureomycin and chloramphenicol. Strains which became resistant to Aureomycin or Terramycin regularly developed cross-resistance to the other. Some of the organisms resistant to Chloramphenicol developed significantly increased resistance to aureomycin and/or terramycin, and some of the strains made resistant to either Aureomycin or Terramycin developed significantly increased resistance to Chloramphenicol.[25]

It is reported that Chloramphenicol produces a depression of hemopoietic activity which promptly disappears following cessation of therapy.[26-27]

Streptomycin

Streptomycin and Dihydrostreptomycin are active agents against a number of gram-negative bacteria. At the present the

greatest usefulness for streptomycin seems to be in the treatment of tuberculosis. Streptomycin can be placed in a class by itself as it permits the rapid development of resistance by many gram-negative bacilli while the other antibiotics, in general, follow the pattern of penicillin.

Resistance to streptomycin is of two kinds: simple resistance and dependence. Organisms that develop resistance to streptomycin have a similar resistance to dihydrostreptomycin.

Cross-resistance has been found among streptomycin, streptothricin, neomycin and streptolin, but not with all organisms which have been studied.[28]

Terramycin

Terramycin is active against a variety of microorganisms, including both gram-positive and gram-negative types and to some degree against rickettsiae and certain of the viruses. Evidence obtained by two different methods (i.e. single-step selection and serial transfer in increasing antibiotic concentrations) indicates that in the vitro development of microbial resistance to Terramycin is similar to the pattern characteristic of penicillin. Preliminary observations have indicated that acquired resistance to Terramycin was accompanied by decreased sensitivity to aureomycin and that acquired resistance to Aureomycin or to chloramphenicol was accompanied by cross resistance to the other agent and to Terramycin. From studies reported by Kaipainen[29] on twenty-seven different bacterial strains cultured in increasing concentrations of Aureomycin, Chloramphenicol, Terramycin, dihydrostreptomycin and penicillin it was found that organisms developing resistance to Aureomycin, Chloramphenicol, or Terramycin in general showed a simultaneous increased resistance to the other two antibiotics, cross-resistance between Aureomycin and Terramycin being most commonly encountered.

Sulfonamides

The term "sulfonamide" is applied to a group of substances consisting of para-aminobenzenesulfonamide (sulfanilamide) and its derivatives. Although several thousand such sulfonamides have been prepared relatively few have found wide use in clinical medicine. In the early years of World War II the sulfonamide drugs were the only really effective chemotherapeutic agents available for treatment of bacterial infections, but these drugs had several undesirable characteristics. In many patients they produce serious reactions. A number of strains of bacteria are naturally resistant to their action, and in other cases highly resistant strains of bacteria often develop from susceptible species that have been exposed to the sulfonamide drugs, so that a considerable percentage of bacterial infections is not amenable to sulfonamide therapy. Furthermore, even in the case of

susceptible pathogens, the usefulness of the sulfonamides is limited by the fact that their antibacterial activity is lessened when large numbers of bacteria are present or even when dead bacteria, pus, blood (serum) or other products likely to be found in infected wounds are present.[30]

Bacitracin

Bacitracin is an antibiotic obtained from an aerobic strain of B. subtilis and has a considerably narrow bacterial spectrum. Bacitracin is primarily antagonistic to the gram-positive bacteria.

In general, organisms sensitive to bacitracin do not develop resistance to it in vivo with any facility, although it is possible to demonstrate this phenomenon in vitro.[31] When resistant strains are developed, they lose their resistance to the drug rapidly.[31]

Polymyxin B

Polymyxin B is highly active against gram-negative bacteria. Resistance of susceptible bacteria to polymyxin is difficult to induce in vitro in contrast to the ease with which resistance of organisms is induced to streptomycin under similar conditions. The fact that organisms develop resistance slowly or not at all to polymyxin increases the value of this drug in infections due to gram-negative organisms where short term treatments are satisfactory and, furthermore, necessary because of the toxicity of the drug.[32]

Nitrofurans

One of the newest classes of synthetic antibacterial agents, the nitrofurans, has several outstanding characteristics. One is that in vitro it is almost always difficult for bacteria to develop resistance to them, and, if resistance is developed it is very limited. Also, resistance to other agents such as penicillin, streptomycin and the sulfonamides does not affect the resistance of bacteria to the nitrofurans.

This limited resistance developed is not to all other nitrofurans but to some of related chemical structure. On trying to determine just how resistant bacteria differ from the original sensitive strain with the nitrofurans the following facts have been established. Nitrofurazone (Furacin) inhibits glucose and oxygen metabolism of sensitive strains more than of the relatively resistant strains; such resistance to Furacin apparently does not show under anaerobic conditions; there appears to be no difference between cell extracts of susceptible and relatively resistant strains in respect to their ability to reduce Furacin.

Neomycin

Neomycin is active against a wide variety of gram-positive and gram-negative bacteria, as well as acid-fast bacilli. It is in-

teresting to note that tubercle bacilli develop resistance to neomycin at a much slower rate than they do to streptomycin. Tubercle bacilli shown to be completely resistant to streptomycin are sensitive to neomycin.

Carbomycin

Carbomycin is a new antibiotic which does not contain a nitrobenzene group. This antibiotic is effective against many gram-positive bacteria, rickettsiae, certain large viruses, and some gram-negative bacteria. Carbomycin[33, 34, 35] has been found highly effective in vivo and in vitro against gram-positive micro-organisms which had become resistant to one, several or all of the commercially available antibiotics. In vitro and in vivo experiments suggest a lack of cross-resistance with other antibiotics in general use.[36]

No significant evidence of toxicity has been observed at the present time.

Resistance to carbomycin has not been found to be readily developed by bacteria. If, however, resistance is acquired, it probably develops in gradual step-wise fashion.[37]

Erythomycin

This antibiotic is effective against a variety of gram-positive bacteria. For some time staphylococcus enteritis responded to Erythomycin but resistance to this antibiotic develops rapidly and resistant staphylococci have been isolated.[38]

Resistance of micro-organisms to the antibacterial effects of Aureomycin, Chloramphenicol and Terramycin does not appear to be a pressing problem at present; if resistance does develop, little will be achieved by shifting to another antibiotic within this group. These three antibiotics suppress (apparently permanently) the penicillinase mechanisms of certain bacteria which are naturally resistant to the antibacterial effects of penicillin.

At present, bacteriological observations indicate that acquired resistance to one antibiotic may be accompanied by decreased sensitivity to several other antibiotics. Such cross-resistance develops during therapy and may occur with or without the development of superinfection. Cross-resistance between Aureomycin and Terramycin is the most prominent and most common.

It has also been observed that prolonged treatment may also lead to a deveolpment of a mycotic infection. Therefore it seems that the natural conclusion we must draw is that the improper use and abuse of these anti-infective agents will reduce their value in therapy and could conceivably give rise to uncontrollable epidemics in the future.

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THE ADVANCING HORIZONS OF THE ATHEROSCLEROSIS PROBLEM*

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It is indeed heartening to realize that many centers throughout this country and other lands are actively engaged in various phases of the problem of atherosclerosis. This is singularly important for we are all familiar with the almost universal incidence of this malady, the marked paucity of research other than observational research up to a period of 5 or 6 years ago, and the inexorable changes which are transpiring in all of us after we have passed the age of 35. Thus, for a purely selfish motive, it is heartening that many scientists are attacking this elusive problem of atherosclerosis from many different disciplines with a single objective in mind albeit the understanding of and thereby the control of the vascular changes of the ageing process or to paraphrase it, the problem of atherosclerosis.

The studies relating to the problem of atherosclerosis have in general followed certain patterns. These can be divided into certain groups, the first, the observational era where the pathologist reigned supreme. Beautiful work was done in this field and we learned from these men that atherosclerosis primarily involved the intima of the blood vessel, seemingly to break through and disrupt the elastic lamina. The results appeared to be a large collection of a cholesterol-like substance with macrophagus near the area. At times actual hemorrhage was found inside one of these cholesterol collections or atheromata. The atheromata appeared to be located in those areas where the vascular tree sustained the greatest amount of trauma related to the ejection of or the passage of blood. In this descriptive era an attempt to relate the observational information with possible changes in the physiology of an organ was made.

For many years we have been guided by the pathologist in the visualization of functional disturbances in a diseased organ. In recent years there has been a noteworthy effort to conjoin the efforts of the pathologist with the physiologist for a more thorough and complete understanding of disease processes. There is no better example of this than in the field of pathologic changes referable to the pulmonary tree. Actual physiologic studies are now being set up whose aim is the analysis of the significant physiologic alterations following the development of certain pathologic changes in the pulmonary vascular bed.

The pathologists are continuing to contribute knowledge in the over-all understanding of the problem of atherosclerosis. They, however, are the first to recognize that what is seen by the visual microscope may not necessarily indicate what is to be found when newer methods are applied in this field. The electron

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microscope or the distribution of certain organic substances as seen by advancing histobiochemical techniques will influence the observational era in the future.

The era which we have been entering in the past 5 or 6 years is the biochemical era and it is in this sphere that active and relentless research is now being engaged. We have alluded to the deposition of a fatty substance in the intima of the artery as being a part of the observational phase of the development of our knowledge of atherosclerosis. These lipid substances have been found to be cholesterol for the most part and as to be expected intensive work has been done to correlate the amount of cholesterol found in the serum with that found in the arterial wall.

Cholesterol is one of the oldest sterols known and was first described by Poulletier in 1769. It was prepared from gall stones; however, it was not until 1815 that the name of cholesterol was given to the compound being derived from the term bile which is chole and stereos meaning solid, bile solid or cholesterol.

Cholesterol is to be found in all cells in the animal kingdom. It is completely absent from the plant world. The highest concentrations of cholesterol are found in the nervous tissue, in the liver and in the fat deposits.

Most of the tissues studied in mammals contain about the same concentration of cholesterol as the corresponding tissues of man, however, the human blood plasma has a higher cholesterol content than that of any other known species. The majority of the lower forms of animals contain a cholesterol concentration in their serum ranging between 30 and 70 mg. % while the mean plasma cholesterol in man is 194 mg.%. The level of plasma cholesterol appears to rise progressively from birth to middle age and then to decline slightly thereafter. In certain pathologic states in which the serum cholesterol is markedly elevated the incidence of atherosclerosis appears to be very high. Experimentally atherosclerosis can be produced in rabbits and fowls by prolonged administration of cholesterol, and the cholesterol and cholesterol esters are increased in the intima and the media of the aorta particularly at the sites where trauma occurs. And thus, one can see from all of the direct and indirect evidences presented, the observational data, the measurement of serum cholesterol, the identification of the lipid material in the intimal wall, all these, tend to incriminate cholesterol in the problem of atherosclerosis. However, when we analyze critically the levels of serum cholesterol with the incidence of atherosclerosis in the human being a direct relationship does not appear to exist. True, there is a rough correlation but not the direct linear relationship that one would expect if there were a simple cause and effect relationship. Thus, since 1916, when Small reviewed the role of cholesterol in the development of atheromatosis up until 1950

the controversy existed without an apparent solution.

Because of the strong implications that had been made in the relationship between atherosclerosis and cholesterol metabolism and with the use of radio-active isotopic techniques it was possible to develop fundamental information as to the factors regulating and transporting the plasma cholesterol. From this work it became apparent that the ingestion of cholesterol was not the primary source of the serum values. In the body, cholesterol may be synthesized from short chain fatty acids as acetic, pyruvic, butyric, hexanoic, octanoic and isovaleric acids. In the rats, squalene has been found to be the intermediate substance isolated as the immediate precursor of cholesterol. A substance of a higher homologue, hepene, has been detected in the human liver. With radioactive carbon it has been found that the synthesis of cholesterol can occur in almost every organ or tissue but the controlling factor for the plasma level of cholesterol has evaded all investigators. Cholesterol is excreted into the intestines although the plasma level is not particularly influenced by this route of excretion. Cholesterol appears to be destroyed primarily in the liver where it is converted into cholic acid and is excreted as one of the bile acids. The serum value of cholate rises prior to the elevation of the serum cholesterol in experimental biliary obstruction and in those clinical states in which hypercholesteremia exists. Thus, the role of surface acting agents such as cholate becomes a factor for future appraisal and experimentation. The serum cholate furthermore appears to alter the plasma so that the liver does not remove cholesterol as rapidly as usual.

The physical-chemical state of the transport of cholesterol in the plasma has interested other investigators for the past few years. It has been found that cholesterol is transported in the serum by attaching to certain protein moieties, the dominant ones being the alpha and the beta globulin. It was noted that very little free cholesterol was found in the circulating plasma. Thus, the state of the plasma proteins influences the cholesterol transport system. These conjugated lipids, the lipo-proteins, have been intensively studied by the use of the ultracentrifuge. Dr. Goffman and his group in California were the first to recognize an increase of certain lipo-proteins in the serum of patients with clinical atherosclerosis. Much has been derived from these contributions, yet, much remains to be found concerning these lipo-proteins.

From the preceding discussion one would assume that either because of an alteration in the transportation of cholesterol in the serum or an alteration in its physical-chemical state the traumatized intima such as found in the aorta would become laden with cholesterol. If atherosclerosis represents a metabolic derangement in either the transport of or the physical-chemical state of cholesterol in the serum one would anticipate that all

the arterial walls in the body would share in a generally uniform fashion. Furthermore the above concept neglects the possibility that an alteration in the intima might result in a local increase of cholesterol irrespective of the conditions existing in the circulating plasma. It has been mentioned earlier that all tissues of the body are capable of synthesizing cholesterol. Chaikoff and his group demonstrated that the aorta in vitro was capable of synthesizing cholesterol in a sizeable amount.

Since atheromata develop in the intima which is made up of connective tissue, it has been the work of our laboratory to study the various chemical constituents of connective tissue. We have devised a technique for the procurement of "pure" connective tissue. Small squares of surgical plastic sponge are embedded in the loose areolar tissue in the posterior aspect of the experimental animal. Connective tissue courses through the pores of the sponge and after 14 days completely fills the sponge. The sponge-fibroblast biopsy can then be removed and analyzed for various histologic and biochemical features. By removal of the sponge at differing times, it is possible to get a biopsy made up primarily of fibroblast, or of fibroblasts with collagen or even fibroblasts with collagen and elastin. It is the hypothesis of our laboratory that connective tissue when injured will elaborate lipid material, cholesterol, and that atherosclerosis may have as its origin the injury of the intimal connective tissue. It is known that patients may have atherosclerosis of the peripheral arteries to rather marked degree and yet the pulmonary bed be entirely free from these deposits. Likewise patients with pulmonary hypertension such as individuals with long standing congestive heart failure or individuals with mitral stenosis may have marked atherosclerotic changes in their pulmonary artery beds with normal systemic arteries.

Connective tissue is a lipid-rich structure. In some of our experiments connective tissue of rats may have fatty acid concentrations as high as the liver and total cholesterol concentrations higher than that found in the liver. The connective tissue in some of the older animals contains a cholesterol concentration exceeded only by that found in the brain or the central nervous system.

Because of the above findings, the connective tissue of rats, rabbits and humans is being studied in our laboratory in the following manner: The lipo-proteins are isolated and separated into alpha and beta fractions. The total fatty acid, the phospholipid, the lecithin and cephalin, the cholesterol, cholesterol esters are determined in each fraction. The protein is then hydrolyzed and chromatographic separation of the amino acids made. The protein of the residue and the lipids of the residue are analyzed. When possible the serum of the animals is studied in a manner

similar to the tissue. This work is now in progress and will be the subject of later reports.

Thus we can see the various eras through which we have passed and are passing in our search for knowledge referable to this universal problem. It is most encouraging to see such a diversified range of scientific disciplines concentrating on this problem. Various eras in the management of the problem of atherosclerosis can be discerned. Approximately 12 years ago spoiled sweet clover was administered to patients in the post-operative period to reduce the incidence of thrombo-embolic phenomenon. Shortly thereafter Dr. E. Sterling Nichol in Miami and Dr. Irving Wright in New York administered Dicumarol to patients with coronary artery disease with the hope and the thought that this would reduce the incidence of subsequent intravascular thrombosis. About two or three years ago some workers in England reported that platelet stickiness was reduced in individuals receiving long term anti-coagulation therapy. Dr. Wright's laboratory reported a change in the zeta-potential of the vascular wall with the long administration of Dicumarol. The total picture of long term anti-coagulation is not known at this time but it does represent an interesting medical approach to the problem. Such work should be encouraged and careful follow-up studies should be made on these patients.

In this regard it should be mentioned that Heparin was found to alter the various lipo-proteins found in the sera of patients with atherosclerosis and it was also noted that Heparin would reduce the post-alimentary hyperlipemia. It was considered for a period of time that Heparin would be of value in the long range picture of patients with atherosclersis. However, more recent work indicates that Heparin only alters larger macromolecules of lipoprotein material to smaller lipo-proteins which then can be metabolized by the body.

I like to believe that atherosclerosis at least at some stage is potentially reversible and I think that this is more than an idle dream. It has been noted by our obstetrical friends that firm tortuous uterine arteries may become soft and pliable with pregnancy. Some of the vascular lesions seen in the pulmonary bed in experimental animals and also in humans are thought to undergo changes following the opening of their narrowed mitral valve. This is the direction towards which all of the basic investigative work is being made. Because of the low incidence of coronary artery disease in women prior to their menopause and because of the beneficial and protective effect of estrogens on experimental atherosclerosis in the chicks some clinicians are giving estrogens to patients with coronary artery disease. It is much too early to formulate any sort of an opinion as to what will be the destiny of this course of medical management.

Our surgical conferees have also been busy to improve the outlook of patients with atherosclerosis. Numerous surgical procedures have been devised for the management of coronary insufficiency. Perhaps the oldest operation for this malady is the so-called cardiopexy in which magnesium trisilicate is dusted into the pericardial space and an adhesive pericarditis results. This seems to relieve coronary insufficiency pain in a number of patients. It is not known whether the life expectancy of these patients is changed to any degree. Dr. Beck and his group in Cleveland have devised the operation of sending arterial blood to the coronary sinus and through the venous system of the coronary bed. This ingenuous approach did not prove too successful for it entailed two surgical procedures and very often the graft made between the aorta and the coronary sinus thrombosed. The Toronto group are using a surgical procedure for coronary insufficiency to provide more arterial blood to the ischemic myocardium by the passage of the internal mammary artery into the left ventricle. This operation has had too limited a period of trial for any definitive comment to be made as to its value.

Accelerated by the traumatic injuries to large and major vessels seen as war time wounds the vascular surgeons have been attacking various lesions of the aorta with considerable success. Aneurysms of the aorta may now be resected. Aortas procured at the autopsy table under sterile conditions are now being lyophilized and preserved indefinitely in a condition ready for immediate use. The lyophilized artery is placed at the site of the resected aorta and a good union made. These grafts have proven to be quite successful in the management of a previously hopeless situation.

I believe we can see from this short review today the tremendous amount of effort that's being directed towards this problem which just a few years ago was thought to be a hopeless scientific imponderable. With the advent of the ultracentrifuge, the introduction of physical-chemical studies, the procedure for studying connective tissue, all research tools poised to contribute an ever increasing volume of information concerning this number one problem. The ingenuousness of the surgeons and the advancement of cardiovascular surgery presents an exciting and stimulating vista into tomorrow. Truly we are in a position to discuss the expanding horizons of the atherosclerosis problem.

HEMATOLOGY

PRESENCE OF ANTI-D ANTIBODY in the Serum of a D^u Patient. C. I. Argoll, J. M. Ball, and E. Trentelman (Utah State Dept. of Health). J. Lab. Clin. Med., 41, 895-8 (1953).
Anti-D antibody was demonstrated in the serum of a D^u obstetrical patient. The question of how to classify D^u patients for the benefit of the clinician is thus raised. As it appears now, any D^u donor and D^u husbands whose wives are D negative should be considered Rh positive and D^u pregnant women as Rh negative.

THE ANTIGLOBULIN (Coombs) TEST as an Aid in the Diagnosis of Hemolytic Syndromes. Robert L. Hare, Frank J. Heck, and Don R. Mathieson (Mayo Foundation Clinic, Rochester, Minn.). J. Lab. Clin. Med., 43, 867-873 (1954).
Eleven patients out of twenty-one were diagnosed as having idiopathic acquired hemolytic anemia. Four gave positive results with the antiglobulin tests using six serums. The variability in the strength of Coombs' serums was demonstrated strikingly. The prozone reaction was shown. That false positive antiglobulin reactions may be caused by cold agglutinins even when the samples of blood are kept at 37° C. was noted. Also the Donath-Landsteiner antibody may sensitize erythrocytes to antiglobulin serum. Technical invalidating errors were suggested such as inadequate washing of erythrocytes, inaccurate preparation of suspensions of erythrocytes and centrifugation at too great a speed.

SOME OBSERVATIONS IN POLYCYTHEMIA VERA. William Dameshek (Tufts College). Bull. New Eng. Med. Center, 16, 53-63 (1954).
Dr. Dameshek's discussion of polycythemia vera noted the intense itching symptoms after bathing, the varied blood picture and its physiology. The myelosclerosis with myeloid metaplasia of the spleen with gradually increasing fibrosis of the marrow in the state called "burnt-out" polycythemia may have a leukocyte rise to levels of 25,000 and platelets of 1.0, 2.0, 5.0 million per cubic millimeter or more with early white cells and occasional nucleated red cells. Some cases show in addition to a very large spleen a "leuko-e·ythroblastic anemia." He suggests that polycythemia vera, chronic granulocytic leukemia, myeloid metaplasia, the DiGuglielmo syndrome and thrombocythemia may be variants of a generalized disturbance. He likes to consider them all as "myeloproliferative disorders" with marrow cells proliferating in one or several directions, thus producing apparently different pictures at different times or retaining its individuality throughout as in some cases of chronic granulocytic leukemia or thrombocythemia. This unified concept for an apparently diverse group of diseases, although not proved, has been of great value in understanding these disorders.

REMARKABLE FAMILY with the Rare Human Isoantibody Anti-Tj^a in Four Siblings: Anti-Tj^a and Habitual Abortion. Shoei Isehi, Shinju Masaki, and Philip Levine (Gunma University, Japan and Ortho Research Foundation, New Jersey). Nature 173, 1193-94 (1954).
Study of the blood of a 27 year old woman and her three compatible siblings were made because of a history of six pregnancies which ended in abortions at two to five months. The serums of this group A, Rh-positive patient and the three siblings agglutinated the blood of fifty random bloods of group O. They did not react with their own red cells, and the red cells of the Virginia Patient in whose serum Levine et al, described the first example of anti-Tj^a. No reaction took place with the red cells of the siblings with three stock samples of anti-Tj^a. There are now thirteen examples of anti-Tj^a in seven families over five continents.

BACTERIOLOGY

CRYSTAL VIOLET BINDING CAPACITY and the Gram Reaction of Bacterial Cells. J. W. Bartholomew and Harold Finkelstein (Univ. of Southern Calif.) J. Bact., 67, 689-91 (1954).
Crystal violet uptake by bacterial cells with quantitative methods did not correlate their gram character. The mechanism of the gram stain as outlined by other investigators was discussed. The authors believe that the date presented merely show the effect of pH and competitive ions on dye retention but very little effect as far as the mechanism of gram differentiation is concerned. Experimental factors were given to substantiate this: (1) iodine cannot precede the dye in the staining procedure: (2) all oxidizing agents cannot replace idodine: (3) decolorizers other than acetone give good gram differentiation: (4) a mucoprotein with an isoelectric point of 1.8 was gram-negative; (5) rupture of cell wall changes a gram-positive cell to a gram-negative state. Dye retention ability has often been confused with dye uptake ability.

THE EFFECTS ON BIOLOGICAL MATERIALS of Freezing and Drying by Vacuum Sublimation. I. Development and Testing Apparatus. Donald Greiff, and Henry Pinkerton (St. Louis Univ.). J. Exp. Med., 100, 81-8 (1954).
Such factors as the speed of freezing, and time, rate, and degree of drying on the survival of bacteria during lyophilization was considered and an efficient and versatile vacuum sublimation apparatus was described. This apparatus will permit (a) the removal of water from virus suspensions at temperatures ranging down to —80° C.,

(b) continuous operation with a minimum of attention from the investigator, (c) sealing off of samples at operating pressures, (d) simultaneous lyophilization of aliquot samples at different temperatures, (e) isolation of a portion of the apparatus without disturbing the remainder of the system, and (f) determination of the end-point of sublimation without disturbing the samples. The time required for drying 0.1 ml. of influenza virus suspension was shown to increase markedly with the decrease of temperature, 8 days being required for dehydration at —80° C. in contrast to 2 days at —30° C. and 1 day at 0° C.

THE EFFECTS ON BIOLOGICAL MATERIALS of Freezing and Drying by Vacuum Sublimation. II. Effect on Influenza Virus. Donald Greiff, Herman Blumenthal, Masahiro Chiga and Henry Pinkerton (St. Louis Univ.). J. Exp. Med., 100, 89-101 (1954).

Procedures involving cyclic slow freezing and thawing, freezing at various rates with subsequent storage at different temperatures, freezing at various rates with subsequent dehydration at various temperatures and different degrees of dehydration were found to influence the survival rate of the virus particles. The effect of lyophilization depended on the preliminary treatment and the dehydration temperature. Possible explanations were suggested, based on known physio-chemical phenomena such as supercooling, vitrification, variations in size and shape of ice crystals with different freezing speeds differential enzyme inactivation, changes in salt concentration, and changes in energy levels.

BIOCHEMISTRY

LEVELS OF 17-HYDROXYCORTICOSTEROIDS in Body Fluids. Avery A. Sandberg, Kristen Eik-Nes, Don H. Nelson, and Frank H. Tyler (Univ. of Utah). J. Lab. Clin. Med. 43, 874-79 (1954).

Determination for free 17-Hydroxycorticosteroids was made without preservatives. Blood was collected with heparin (4 mg. per 30 ml.). The method used was a modification of the method of Nelson and Samuels. Conjugated steroids are not measured by this procedure. Normal subjects showed no 17-Hydroxycorticosteroids in the spinal fluid. In pathologic states especially in those where the meninges were inflamed the 17-Hydroxycorticosteroids appeared in the spinal fluid. Pleural, ascitic, and pericordial fluids showed significant amounts.

HISTOLOGY

THE PREPARATION OF LARGE HISTOLOGIC SECTIONS. A. H. Oakley and J. W. Miller (St. Bartholomew's Hospital, London) Tech. Bull. 24, 176-80 (1954).

Sections of lantern-slide size were prepared by a simple method without the use of the vacuum embedding bath. Ten years of successful use. An abbreviated outline entails these steps:

1. Fix slices of tissue 5 to 10 mm. thick in 10 per cent formal-saline for 24 hrs. Hold air-containing tissues under fixative by mechanical means.
2. Trim slices to 5 mm. and fix in formol-saline 24 hours.
3. Decalcify when necessary in Perenyi's fluid.
4. Dehydrate from formal-saline or Perenyi's fluid *directly* into
   70 per cent alcohol, 24 hrs.
   90 per cent alcohol, 24 hrs.
   100 per cent alcohol, (1), 3 hrs.
   100 per cent alcohol, (2), 3 hrs.
   100 per cent alcohol plus anhydrous copper sulfate, 2 hrs.
   (cover tissue with filter paper, cover filter paper with anhydrous copper sulfate to a depth of 10 mm. to prevent direct contact with tissue)
5. Clear in:
   Chloroform, (1), overnight
   Chloroform, (2), 1 hr.
   Xylene, 15 minutes
6. Impregnate in:
   Paraffin wax of melting point 56° C. (or if crystallization occurs use 1 part of wax with melting point of 54° C. and 2 parts of wax with melting point of 56° C.) for a total of 7 hrs. (4 changes in the first hour, then change every 2 hours.)
7. Embed in paraffin wax.
8. Trim paraffin block closely, leaving a small margin on side parallel to knife. have base as flat as possible and remove surface wax to expose tissue.
9. Attach block to microtome holder.
10. Immerse block and base in cold water for ½ to 1 hour, after complete surface of tissue is exposed.
11. Make final section with a precision, sliding microtome 5 to 10 μ thick (Spencer make used by authors).
12. Float section onto warm water about 5 degrees below melting point of wax.
13. Float sections onto clean lantern slides and gently but firmly blot dry with "fluffless" blotting paper. (Occasionally a thin film of blood plasma may be used as an adhesive.)
14. Place slides on trays and incubate at 40° C. overnight.
15. Employ usual methods for staining and adequate balsam in mounting.

MISCELLANEOUS

EFFECTIVE TESTIMONY FOR SCIENTIFIC WITNESSES. Wilmer Souder (National Bureau of Standards, Washington, D. C.) Science 119, 819-822 (1954).
Experiences are summarized over a thirty year period of presenting scientific findings in Federal Courts. The position and attitude of the scientist in presenting his findings is explained. The courtroom atmosphere, the audience—especially the jury—and the cross examination of the attorneys are noted.

ARMY MEDICAL MUSEUM AND ARMED FORCES INSTITUTE OF PATHOLOGY IN HISTORICAL PERSPECTIVE. Morris C. Leikind (Armed Forces Institute of Pathology, Washington, D. C.) Sci. Monthly 79, 71-8 (1954).
The history of the Medical Museum established in 1862 until July 6, 1949, when the Armed Forces Institute of Pathology was established is given in detail. More than 150,000 visitors per year indicates the drawing power of the museum with its important scientific assemblage of material.

ARMED FORCES INSTITUTE OF PATHOLOGY: RETROSPECT AND PROSPECT. Hugh G. Grady (Armed Forces Institute of Pathology, Washington, D. C.) Sci. Monthly 79, 79-80 (1954).
The fundamental interest in the morphology and behavior of diseases affecting military and civilian populations will be continued in the new facilities of the AFIP. Each senior member of the staff will have a basic laboratory unit, and, in addition, there will be facilities for work in histochemistry, electron microscopy and radiobiology.

NEW BUILDING FACILITIES FOR ARMED FORCES INSTITUTE OF PATHOLOGY. Colin F. Vorder Bruegge (Armed Forces Institute of Pathology, Washington, D. C.) Sci. Monthly 79, 81-9 (1954).
Detailed descriptions and picture diagrams are given of the new building of AFIP located on the grounds of the Walter Reed Army Medical Center. Laboratory counters are "stand-up height" (37 in. from the floor) and "sit-down height" (31 in. from the floor). Albarene stone is used for most of the laboratory sinks and this or synthetic stone is used for counters in the chemistry-type laboratories. Birch countertops with black acid-resistant finish are used in the majority of general-purpose laboratories. Stainless steel counters are furnished in several rooms designed for work with radioactive isotopes or dangerous living organisms. Especially designed air-conditioning systems are installed. A modest, closed-circuit color television system is included.

AMONG THE NEW BOOKS

A MANUAL OF TROPICAL MEDICINE: 2nd Ed. by Thomas T. Mackie, M.D., Colonel, M.S.C.A.U.S. (retired), Chairman, The American Foundation for Tropical Medicine; George W. Hunter, III, Ph.D., Colonel, M.S.C., U.S.A., Chief, Section of Parasitology-Entomology, Fourth Army Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston, Texas; C. Brooke Worth, M.D., Field Staff Member, Division of Medicine and Public Health, The Rockefeller Foundation. Philadelphia and London; W. B. Saunders Company, 1954. 907 pages. 304 illustrations, 7 in color. $12.00.

This edition has been brought up to date by drawing from recent medical and technical journals as well as specialized texts. Based originally upon military medicine, the increased knowledge of epidemiology, treatment, and control of preventable, endemic, and communicable disease is of vast importance to the general economy. This volume contains much new information due to a shifting emphasis on many of the diseases considered. For the medical technologist this background material is essential. There are sections on virus, rickettsial, spirochetal, bacterial, mycotic, protozoal, and nutritional diseases. Most have detailed notes on definition, distribution, etiology, epidemiology, pathology, clinical characteristics, and diagnosis. Some include prognosis and treatment as well. There is a chapter on special laboratory diagnostic methods beside the invaluable discussions throughout the book—all of which can be well-taken by the technologist.

ISOTOPIC TRACERS: A Theoretical and Practical Manual for Biological Students and Research Workers, by G. E. Francis, Reader in Biochemistry, St. Bartholomew's Hospital Medical College, London England; W. Mulligan Senior Lecturer in Biochemistry, Glasgow University Veterinary School; and A. Wormall, Professor of Biochemistry, St. Bartholomew's Hospital Medical College, London, England.

London. The Athlone Press. Distributed by John de Graff, Inc., New York. 1954. 306 pages, 51 figures, 7 tables. Appendix. $7.00.

This book is divided into two parts. The first part gives something of the general principles of isotopic tracer techniques, including a chapter on atomic structure; radioactive and stable isotopes, their preparation and synthesis. There is also a chapter on the hazards and precautions in the use of radioactive isotopes.

The second part is a Practical Course, including chapters on the use of Gieger Muller tubes for the measurement of radioactive isotopes. Other exercises are devoted to various determinations. The fifteen appendices include a glossary of terms, preparation of special equipment, reagents for testing, and methods of expressing data. With the incorporation of these techniques into the clinical laboratory, this volume will be of increasing value.

THE MICROTOMIST'S FORMULARY AND GUIDE: by Peter Gray, Ph.D., D.I.C., F.R.M.S., Head, Department of Biological Sciences, University of Pittsburgh. The Blakiston Company, Inc., New York, Toronto, 1954. 794 pages, 86 illustrations. $10.50.

An extremely useful part of every histology laboratory library, and of more than average value for the teaching laboratory, the techniques in making various types of mounts are described in detail. Preparation of other types of material is also illustrated in detail. Part two covers the subject of methods and formulas used in making microsope slides: fixatives, dye stains of general and special application, metal stains, and mounting and embedding media are all noted. Finally there is a fairly complete listing of books cited in the pages preceding.

LABORATORY AIDS IN ENDOCRINE DIAGNOSIS: by Roberto F. Escamilla, M.D., Associate Clinical Professor of Medicine, University of California Medical School, San Francisco, Calif. Springfield, Illinois, Charles C. Thomas, Publisher, 1954. 131 pages, 21 illustrations. $4.75.

Although "pocket size," this will become one of the "routine" laboratory stand-bys. In a short chapter the common tests required in endocrine disorders are mentioned. More detail is allowed for the special tests—blood, urine, gastric analysis, and even the newer radioactive determinations are described. Sufficient detail is included to make an adequate laboratory appraisal although further background material would be required for more thorough treatment.

HISTOPATHOLOGIC TECHNIC AND PRACTICAL HISTOCHEMISTRY: by R. D. Lillie, M.D., Medical Director, U. S. Public Health Service; Chief, Pathologic Anatomy Service, Clinical Center, National Institute of Health, and Chief, Laboratory of Pathology and Pharmacology, National Institute for Arthritis and Metabolic Diseases. New York. The Blakiston Company, Inc., 1954. 591 pages, 32 tables. First Ed. published under title of HISTOPATHOLOGIC TECHNIC. $7.50.

A very practical volume for the teaching laboratory gives the fundamentals of microscopy and describes the equipment before going into the details of technique from fixation and sectioning to stains, staining, and mounting methods. More than half the volume is devoted to histochemistry with specific technics for handling and staining. Bacteria, protozoa, and other parasites are included in the general study.

CHEMOTHERAPY OF INFECTIONS: by H. O. J. Collier, Ph.D., M. I. Biol., Chief Pharmacologist, Allen and Hanburys Ltd. New York. John Wiley & Sons, Inc., 1954. 247 pages, 53 illustrations and diagrams, 21 tables. $4.00.

Valuable primarily as background information, this can be used as a handbook on the effects of the newest drugs on the tissues and infective organisms involved. The comparative effects of these antibiotics are evaluated and described from the chemical rather than the bacteriological viewpoint.

LECTURES ON THE SCIENTIFIC BASIS OF MEDICINE, Vol. II. British Postgraduate Medical Federation. New York and London. John de Graff, Inc., The Athlone Press, 1954. 380 pages, 29 plates. $6.00.

This collection of nineteen lectures is the result of a review of the fields of research in which there have been notable advances in the knowledge of fundamental principles that may later be applied to a better understanding of health and disease. This is the gist of the Preface as stated by Professor Sir Francis Fraser, M.D. F.R.C.P. The lecture series must be considered "planned as a stage in an integrated, continuously developing survey, which can only yield its full value to the student who keeps himself continuously familiar with it." The volume, therefore, serves as background material for the current and future developments in the field of clinical medicine. Of practical value to the technologist are those lectures dealing with the "Preservation of Living Cells at Low Temperatures," "The Life Span of Red Blood Cells," and "Human Haemoglobins."

HISTOLOGY: Edited by Roy O. Greep, Ph.D., with thirteen contributors. Harvard Medical School. New York. The Blakiston Company, Inc., 1954. 953 pages. 648 illustrations, many in color. $15.00.

The general approach to the subject differs from the usual, with morphology of paramount concern. From this point of view an attempt is made to make the nature of cells and tissues better understood. There are four general chapters on the Structure of Organisms, Cell Structure and Function, Histochemistry, and Embryological Origin of Tissue. Each chapter following treats a different tissue specifically. Profusely and beautifully illustrated, this text will be an addition to the reference library of any histology department.

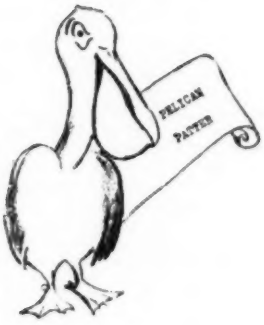
ANTIBODIES AND EMBRYOS: by W. F. R. Brambell, W. A. Hemmings, and M. Henderson, University of London. London, Athlone Press. Distributed by John de Graff, Inc., New York, 1954. 103 pages, 15 tables, 10 illustrations. $2.25.

This series of lectures describes recent research on the passage of antibodies from the mother to the foetus in rabbits. The fundamental problem involved is the mechanism of transmission of proteins across living membranes. There is a chapter on the passive transfer of immunity discussing the transmission both before and after birth. These reports cover research conducted on rabbits primarily, but the chapter dealing with Immunity and Survival concerns the development of hemolytic jaundice in mule foals.

ALSO RECEIVED:

TEXTBOOK OF BIOCHEMISTRY: Sixth Ed. Benjamin Harrow, Ph.D., Professor of Chemistry, College of the City of New York; and Abraham Mazur, Ph.D., Associate Professor of Chemistry, College of the City of New York. Philadelphia and London. W. B. Saunders Company, 1954. 563 pages, 131 figures. $6.00.

THE FUNDAMENTALS OF X-RAY AND RADIUM PHYSICS: by Joseph Selman, M.D., Chief of Radiology, Mother Frances Hospital, Director Radiology Department, Medical Center Hospital, Tyler, Texas. 1954. Charles C. Thomas, Springfield, Illinois. 340 pages. 174 figures. 8 tables. $8.50.



The 1954 convention is over—long live A.S.M.T. Conventions! Florida gives way to Louisiana and Patate the Pelican takes over from Seminole Sally. After due consultation with this state bird—our host next year—he has agreed to write for you in each issue a column (Pelican Patter) about the likely doings in New Orleans next June. But, first let me tell you how all this came about—

As the moon went down over Miami on Thursday night June 17, the twenty-second annual convention of the American Society of Medical Technologists came to a close. Those of us who had the pleasure of attending this convention will always remember the fabulous year-round playground of the Americas, Miami Beach. A vivid picture of the numerous luxurious hotels, its colorful cabana colonies, beautiful beaches and view of the ocean will always remain with us.

Our hosts, members of the Florida State Society of Medical Technologists, with identification badges of colorful Seminole Indian dolls were easily spotted to assist with our problems. Their geniality, enjoyable entertainment, and excellent scientific programs which they planned will be remembered with appreciation.

As we wended our way back home through the Gulf States, our thoughts were on the 1955 convention which is to be held in New Orleans.

But—as we crossed the Pearl River Swamp and set foot again in our own state—we stopped—suddenly gloomy—to take a look around. Lagoons and bayous studded with water hyacinths and wild iris and fat white lilies spread all about in a restful haven of serenity. The tall marsh grasses waved invitingly.

Pretty?—yes—but what to take the place of the Florida charms in producing another fine gay and unforgettable convention? We sat on a nice old cypress stump and faced the problem—we concentrated on our state—we closed our eyes and said "Louisiana"—we opened them—quick—and out of the marshes—full of herons and egrets and gulls—came old man Louisiana himself—a big fat, brown pelican (Patate, the Pelican).

He turned his big long yellow beak sideways—habitually quizzical—and waited. We stated our problem—we wanted our state—our whole state—to have just as gracious, hospitable, entertaining and instructive a convention as Florida.

"Eh bien," he said, promptly (this old Pelican comes from the southwestern part of the state—the "Cajun"—bayou country—and lately he has lived nearer to Creole New Orleans—so that sometimes when you hear him—you think you're maybe in Paris—yes?) "C'est facile! that's easy!"

"You know why, yes, they call us the Pelican state, eh—NO? Well, they used to say the pelican mère—she tear her breast to feed her young—so Louisiana she do anything—anything, Ma Chère—to do for her people—so you come right in asking Patate the Pelican, to help you." He winked his lugubrious eye.

"We make a fine convention—you going to see."

"Your friends come to Louisiana next year—what they think of? Well, some—they dream of jasmine and magnolia and honeysuckle making sweet the star studded nights—and mocking birds and cardinals making the whole night to sing, and live oaks and Spanish moss—waving 'doucement'—and delicate crepe myrtle and hibiscus. Yes, some think of that but some too," he shrugged, "think of—oil wells and natural gas—big money!—and rice fields—more than any other state—that goes for sulphur, too, and even salt mines—N'est-ce-pas—and then some maybe think of romance and ante bellum mansions and wrought iron lace work balconies and gardenia-studded courtyards and candle-lighted patios—those are the romantics—maybe some think of Ole Man Mississippi river and the levees and steamboats and ocean-going liners and tramp ships—the second port in the country—you know—maybe they like to see the International House and the Trade Zone—ah, yes, maybe they will—*but* they're coming to New Orleans—and all of them—'Mais oui—toute le monde.' I mean everybody will think of Oysters' Rockefeller, Ramos gin fizz, and boullabaisse and Shrimp Arnaud and absinthe frappé and crepe Suzettes—um! and cherries jubilée and crawfish bisque—ou-là-là."

"So bring on your friends—we make them a big time! Ma chère—you plan—the what you call it—the scientific part—eh bien! that will be easy—one of the biggest and most up-to-date medical centers on earth right here by us—and we'll take care of their fun. We'll make them understand why they call New Orleans the city that care forgot!"

"But more next time!!!"

Au revoir,
Patate le Pelican

SMITHSONIAN INSTITUTE INSTALLS EXHIBIT FROM BECTON, DICKINSON SHOWING HISTORY OF HYPODERMIC SYRINGE

Antique and modern hypodermic syringes and needles have joined "The Spirit of St. Louis," old "iron horses" and other classic examples of human experience in the Smithsonian Institute.

A permanent historical exhibit prepared by Becton, Dickinson and Company shows how the present instrument has evolved.

The exhibit is an illuminated wall panel with a giant model of the B-D Yale Luer-Lok syringe in the center. It is displayed in the Institute's Pharmacy Division.

"The Story of Hypodermic Syringes" includes many kinds used in medicine today and also describes the steps in their manufacture, as well as their history.

Especially fascinating as medical artifacts are the early "syringes" that had no needles. Their elongated nozzles were inserted into previously made incisions, and the medicinal ingredients such as morphine paste were deposited there. Some of these "syringes" were made of sterling silver with leather pistons.

Sir Christopher Wren, the exhibit points out, was probably the first to use a syringe to inject drugs into the body. In 1657 he produced an instrument by attaching a slender quill to a bladder. But it was not until 1852, almost two centuries after Wren, that Charles-Gabriel Pravaz of

Lyons, France, devised a metal hypodermic syringe.

Charles Hunter of London introduced the cutting point on the needle in 1859. First to patent a hypodermic syringe in the United States, in 1873, was Ephriam Cutter of Woburn, Mass.

Hermann Wulfing Luer of Paris revolutionized the hypodermic syringe by developing the first all-glass instrument. It was first manufactured in the United States by Becton, Dickinson and Company, in 1897. The first sale was made by M. W. Becton to Z. D. Gilman of Washington, D. C. on October 8 of that year, and the price was $60.00 per dozen.

In 1925, Col. F. S. Dickinson designed the Luer-Lok syringe, the first with a practical form of needle and syringe joint. It is in common use today and has largely replaced the earlier types of metal cylinder-leather piston types of syringes.

CONTINUATION COURSE

The University of Minnesota announces a continuation course in Clinical Microscopy and Parasitology for Medical Technologists. The course will be held at the Center for Continuation Study on the University campus from November 15 to 17, 1954. Half of the three-day program will be devoted to the examination of various body fluids such as urine, spinal fluid, and gastric secretion; the remainder to parasitology. Guest faculty will include Dr. Donald B. McMullen, Chief, Department of Medical Zoology, Army Medical Service Graduate School, Washington, D. C. Lodging and meal accommodations are available at the Center for Continuation Study. Write to Dr. Robert B. Howard, Director Department of Continuation Medical Education, University of Minnesota Medical School, Minneapolis 14, Minnesota.

ARMY SCHEDULES COURSES IN CARE OF ATOMIC CASUALTIES

The first of four five-day courses to be given by the Army Medical Service during the present fiscal year on "The Medical Care of Atomic Casualties" began on August 24, it has been announced by Major General George E. Armstrong, The Surgeon General.

The courses, endorsed by the Secretary of the American Medical Association and the Secretary of the Council on Emergency Medical Service of the American Medical Association, will be presented at the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

The other three courses to be presented during the fiscal year will be given on the following dates: October 25-30, 1954; January 10-15; and March 7-12, 1955.

The courses are open to active duty professional officers of the component corps of the Army Medical Service and to medical service professional personnel of the Air Force, Navy, Public Health Service, Veterans Administration and Civil Defense.

Army Medical Service personnel should submit applications to the Office of The Surgeon General, Attention: Personnel Division, Career Management Branch, Washington 25, D. C. All others desiring to attend the course should direct their applications through their proper command or administrative channels.

ASMT ADOPTS OFFICIAL GROUP INCOME PROTECTION INSURANCE PLAN

The American Society of Medical Technologists is pleased to announce the official sponsorship of a new group plan of income protection, as unanimously approved by the Board of Directors and the House of Delegates at the June convention at Miami Beach.

The program is designed to provide eligible members with insurance against the loss of income in the event of disability due either to sickness or accident. By taking advantage of the Society's collective purchasing power, this protection is available at costs which are as much as 30 to 50 per cent lower than those usually required for individual policies. Through group purchase, we are able to gain advantages both as to rates and conditions of coverage which could not be had under individual policies.

Because of the wide geographical spread of the membership and to spare members from time-consuming personal solicitation, the enrollment campaign will be conducted almost entirely by mail. It has been arranged with the insurance company selected that if 50 per cent of the membership apply during the initial enrollment period, all eligible applicants will be insured without regard to their present physical condition or past medical history. In order to make sure that every member understands this valuable new service of the Society and has every opportunity to participate in it so that the 50 per cent goal may be achieved, the initial enrollment period will be held open for several months.

This program should be considered a vital part of the economic security program of the medical technologist, a service made available only to ASMT members. It provides for up to two years of weekly cash benefits during periods of disability due to accident, and up to one year of weekly cash benefits in the event of disability resulting from sickness. Such benefits commence with the first day of disability due to accident and the eighth day for loss of time caused by sickness, and paying for any of the first seven days spent in a hospital. Substantial benefits are payable for accidental death and dismemberment, partial disability caused by accidents, and medical treatment for non-disabling injuries.

Among the outstanding features of the program is the provision that no member will be refused the renewal of his policy certificate as long as the ASMT Plan is continued in force by the Society and the insurance company, and as long as premiums are paid while a Society member is in active practice up to the next premium due date after the sixty-fifth birthday.

House confinement is never required to collect disability benefits. The only exclusions are military service, war, operating an airplane, suicide, and pregnancy. Sports and recreations are fully covered, as well as professional duties. Benefits never decrease because of age.

Members have recently received descriptive material and application forms. Since this is the only officially sponsored group insurance program of the Society, it is hoped that many members will take advantage of this Plan to provide for the unforeseeable future. The successful continuing operation of the Plan will be dependent upon a high rate of member participation in it.

The Plan will operate under the supervision of the Insurance Committee of which Miss Barbara Isbell of San Diego, California, is chairman. Other members are Mr. C. Patton Steele of Bismark, North Dakota, and Mrs. Elsa Kumke of Detroit, Michigan.